

This Page Is Inserted by IFW Operations
and is not a part of the Official Record

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

**As rescanning documents *will not* correct images,
please do not report the images to the
Image Problem Mailbox.**

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
16 May 2002 (16.05.2002)

PCT

(10) International Publication Number
WO 02/38805 A2

(51) International Patent Classification⁷: **C12Q 1/68**

(21) International Application Number: **PCT/EP01/13034**

(22) International Filing Date:
9 November 2001 (09.11.2001)

(25) Filing Language: **English**

(26) Publication Language: **English**

(30) Priority Data:
60/246,750 9 November 2000 (09.11.2000) **US**

(71) Applicant (for all designated States except US): **CENIX BIOSCIENCE GMBH [DE/DE];** Pfotenhauerstrasse 108, 01307 Dresden (DE).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **ECHEVERRI,**

Christophe [FR/DE]; Schlossberg 10a, 69117 Heidelberg (DE). **GOENCZY, Pierre** [CH/CH]; 10, avenue de la Harpe, CH-1007 Lausanne (CH). **HYMAN, Anthony** [GB/DE]; Hölderlinweg 14, 69120 Heidelberg (DE). **COULSON, Alan** [GB/GB]; 28 Blinco Grove, Cambridge CB1 7TS (GB). **JONES, Steven** [GB/CA]; 600 West 10th Avenue, Vancouver, British Columbia V5Z 4E6 (CA). **OEGEMA, Karen** [US/DE]; Schlossberg 10b, 69117 Heidelberg (DE). **KIRKHAM, Matthew** [GB/DE]; Ladenburgerstrasse 57, 69120 Heidelberg (DE).

(74) Agent: **ISENBRUCK, Günter;** Bardehle Pagenberg Dost Altenburg Geissler Isenbruck, Theodor-Heuss-Anlage 12, 68165 Mannheim (DE).

(81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC,

[Continued on next page]

(54) Title: **EUKARYOTIC CELL DIVISION GENES AND THEIR USE IN DIAGNOSIS AND TREATMENT OF PROLIFERATIVE DISEASES**

Multiple Sequence Alignment of the H38K22.2a family

```
CeH38K22.2a  ---M---D---T---L---K---V---O---V---T---A---V---S---I---N---F---A---K---A---N---I---E---Y---D---T---L---Y---D---R---N---L---F---A---G---S---T---P---
CeH38K22.2b  ---M---K---K---D---O---
DmCG7427     M---L---O---N---E---S---T---H---A---D---V---V---I---S---L---T---H---T---G---O---T---A---F---C---L---O---C---H---D---K---F---L---E---S---D---N---Y---F---O---N---E---Y---Y---R---E---
HsAAH04863   ---M---K---K---S---O---K---D---V---K---O---D---H---I---F---O---S---S---K---T---A---S---C---S---D---K---L---E---Y---T---O---N---F---O---N---E---Y---Y---R---E---S---V---
HsAAH09478   ---M---K---K---S---O---K---D---V---K---O---D---H---I---F---O---S---S---K---T---A---S---C---S---D---K---L---E---Y---T---O---N---F---O---N---E---Y---Y---R---E---S---V---

CeH38K22.2a  Q---P---S---O---S---N---E---E---F---N---O---Y---V---B---K---D---V---G---E---K---R---G---P---H---E---D---R---L---L---T---D---G---Y---E---A---T---D---R---N---Y---F---O---N---E---Y---Y---R---E---S---T---
CeH38K22.2b  ---K---T---K---E---R---F---N---O---Y---V---B---K---D---V---G---E---K---R---G---P---H---E---D---R---L---L---T---D---G---Y---E---A---T---D---R---N---Y---F---O---N---E---Y---Y---R---E---S---T---
DmCG7427     E---P---K---K---R---F---N---O---Y---V---B---K---D---V---G---E---K---R---G---P---H---E---D---R---L---L---T---D---G---Y---E---A---T---D---R---N---Y---F---O---N---E---Y---Y---R---E---S---T---
HsAAH04863   K---G---S---H---K---K---E---F---N---O---Y---V---B---K---D---V---G---E---K---R---G---P---H---E---D---R---L---L---T---D---G---Y---E---A---T---D---R---N---Y---F---O---N---E---Y---Y---R---E---S---T---
HsAAH09478   K---G---S---H---K---K---E---F---N---O---Y---V---B---K---D---V---G---E---K---R---G---P---H---E---D---R---L---L---T---D---G---Y---E---A---T---D---R---N---Y---F---O---N---E---Y---Y---R---E---S---T---

CeH38K22.2a  C---H---E---S---L---D---E---N---V---K---E---T---A---L---O---A---D---V---O---N---E---R---O---I---D---S---I---N---S---G---E---S---K---A---T---H---E---L---Y---L---E---A---N---Y---S---A---A---C---N---
CeH38K22.2b  D---E---S---L---D---E---N---V---K---E---T---A---L---O---A---D---V---O---N---E---R---O---I---D---S---I---N---S---G---E---S---K---A---T---H---E---L---Y---L---E---A---N---Y---S---A---A---C---N---
DmCG7427     E---S---R---D---F---I---N---G---C---D---L---G---I---S---D---K---T---K---P---I---L---E---O---N---B---A---G---K---D---F---H---I---T---T---N---Y---D---P---G---O---S---G---
HsAAH04863   E---S---K---O---F---H---O---S---T---E---L---G---C---D---L---E---K---I---A---O---E---P---K---M---E---O---E---K---E---P---C---D---F---H---I---T---T---N---Y---D---P---G---O---S---G---
HsAAH09478   E---S---K---O---F---H---O---S---T---E---L---G---C---D---L---E---K---I---A---O---E---P---K---M---E---O---E---K---E---P---C---D---F---H---I---T---T---N---Y---D---P---G---O---S---G---

CeH38K22.2a  E---R---E---T---A---C---C---O---V---L---F---G---O---S---T---I---N---T---O---I---D---I---L---W---A---O---N---A---A---S---L---A---Q---N---V---G---A---S---N---A---K---O---F---K---S---V---W---I---S---E---
CeH38K22.2b  E---R---E---T---A---C---C---O---V---L---F---G---O---S---T---I---N---T---O---I---D---I---L---W---A---O---N---A---A---S---L---A---Q---N---V---G---A---S---N---A---K---O---F---K---S---V---W---I---S---E---
DmCG7427     E---R---E---T---A---C---C---O---V---L---F---G---O---S---T---I---N---T---O---I---D---I---L---W---A---O---N---A---A---S---L---A---Q---N---V---G---A---S---N---A---K---O---F---K---S---V---W---I---S---E---
HsAAH04863   E---R---E---T---A---C---C---O---V---L---F---G---O---S---T---I---N---T---O---I---D---I---L---W---A---O---N---A---A---S---L---A---Q---N---V---G---A---S---N---A---K---O---F---K---S---V---W---I---S---E---
HsAAH09478   E---R---E---T---A---C---C---O---V---L---F---G---O---S---T---I---N---T---O---I---D---I---L---W---A---O---N---A---A---S---L---A---Q---N---V---G---A---S---N---A---K---O---F---K---S---V---W---I---S---E---

CeH38K22.2a  N---I---F---W---I---L---L---S---K---P---O---L---S---D---D---D---S---A---V---E---V---D---D---H---Y---C---R---E---N---L---N---Y---K---P---G---N---S---D---O---O---M---E---T---P---R---I---A---Q---
CeH38K22.2b  N---I---F---W---I---L---L---S---K---P---O---L---S---D---D---D---S---A---V---E---V---D---D---H---Y---C---R---E---N---L---N---Y---K---P---G---N---S---D---O---O---M---E---T---P---R---I---A---Q---
DmCG7427     N---I---L---L---E---A---T---N---I---D---D---R---N---S---N---D---S---E---O---V---E---D---D---H---Y---C---R---E---N---L---N---Y---K---P---G---N---S---D---O---O---M---E---T---P---R---I---A---Q---
HsAAH04863   N---I---L---L---E---S---S---M---I---A---D---D---M---S---N---D---S---E---O---V---E---D---D---H---Y---C---R---E---N---L---N---Y---K---P---G---N---S---D---O---O---M---E---T---P---R---I---A---Q---
HsAAH09478   N---I---L---L---E---S---S---M---I---A---D---D---M---S---N---D---S---E---O---V---E---D---D---H---Y---C---R---E---N---L---N---Y---K---P---G---N---S---D---O---O---M---E---T---P---R---I---A---Q---

CeH38K22.2a  K---K---P---G---I---F---Y---F---N---S---H---L---Q---I---E---F---K---L---F---O---Y---P---M---L---K---T---I---F---K---I---T---I---H---T---A---G---T---N---R---
CeH38K22.2b  K---K---P---G---I---F---Y---F---N---S---H---L---Q---I---E---F---K---L---F---O---Y---P---M---L---K---T---I---F---K---I---T---I---H---T---A---G---T---N---R---
DmCG7427     S---Q---K---H---I---S---S---A---Y---Q---T---S---H---S---T---N---D---N---Y---G---
HsAAH04863   -----
HsAAH09478   -----
```

(57) Abstract: The present invention relates to the significant functional role of several *C. elegans* genes and of their corresponding gene products in cell division and proliferation processes that could be identified by means of RNA-mediated interference (RNAi) and to the identification and isolation of functional orthologues of said genes including all biologically-active derivatives thereof. The invention further relates to the use of said gene products (including said orthologues) in the development or isolation of anti-proliferative agents, particularly their use in appropriate screening assays, and their use for diagnosis and treatment of proliferative diseases.

WO 02/38805 A2

E-value: 1e-49 Identities: 101/275 (36%) Positives: 159/275 (56%)	E-value: 7e-45 Identities: 100/275 (36%) Positives: 157/275 (56%)	E-value: 6e-44 Identities: 104/299 (36%) Positives: 154/299 (56%)	



LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— without international search report and to be republished upon receipt of that report

(84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF,

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

**Eukaryotic cell division genes and their use in diagnosis and treatment of
proliferative diseases**

5

In a first aspect, the present invention is related to the significant functional role of several *C. elegans* genes and of their corresponding gene products in cell division and proliferation processes that could be identified by means of RNA-mediated interference (RNAi).

10 In a second aspect, the invention relates to the identification and isolation of functional orthologues of said genes and their gene products found in other eukaryotic species, in particular man, including all biologically-active derivatives thereof.

In a third aspect, the present invention includes the use of said genes and gene products (including said orthologues) in the development or isolation of anti-proliferative agents for instance their use in appropriate screening assays and in methods for diagnosis and
15 treatment of proliferative diseases.

In a forth aspect, the invention relates to antibodies to said gene products and their use in the development or isolation of anti-proliferative agents and in methods for diagnosis and treatment of proliferative diseases.

20 In a fifth aspect, the present invention is related to the use of these genes and gene products for developing structural models or other models for evaluating drug binding and efficacy as well as to any other uses which are derived from the new functions described here and which will become apparent from the disclosure of the present application for any person skilled in the art.

25 Metazoan cell division consists of an extremely complex, highly regulated set of cellular processes which must be tightly co-ordinated, perfectly timed, and closely monitored in order to ensure the correct delivery of cellular materials to daughter cells. Defects in these processes are known to cause a wide range of so-called proliferative diseases, including all forms of cancer. Since cell division represents one of the few, if not the only cellular
30 process that is common to the aetiology of all forms of cancer, its specific inhibition has

- 2 -

long been recognised as a preferred site of therapeutic intervention. Although mitotic inhibitor drugs are recognised as one of the most promising classes of chemotherapeutic agent, screening attempts to find new drug candidates in this class have been undermined by the strong inherent tendency of such screens to identify agents that target a single
5 protein, tubulin. Tubulin polymerises to form microtubules, the primary cytoskeletal elements needed for mitotic spindle function and chromosome segregation. Microtubule functions, however, are ubiquitously needed in almost all cell types, whether dividing or not, a fact which therefore explains many of the unwanted side effects caused by anti-tubulin drugs.

10

Perhaps the best known example of a highly successful anti-neoplastic drug that targets tubulin is provided by paclitaxel, and its marketed derivative, Taxol, from Bristol Meyers Squibb. Its applicability has indeed been seriously limited by difficulties in determining an adequate dosing regimen due to a range of problematic side effects. Taxol treatment has
15 resulted in anaphylaxis and severe hypersensitivity reactions characterised by dyspnea and hypotension requiring treatment, angioedema, and generalised urticaria in 2-4% of patients in clinical trials. All Taxol is administered after pretreatment with corticosteroids and despite pretreatment, fatal reactions have occurred. Severe conductance abnormalities resulting in life-threatening cardiac arrhythmia occur in less than 1 percent of patients and
20 must be treated by insertion of a pacemaker. Taxol can cause fetal harm or fetal death in pregnant women. Furthermore, administration is commonly accompanied by tachycardia, hypotension, flushing, skin reactions and shortness-of-breath (mild dyspnea).

Despite these shortcomings, Taxol has been hailed by many as the most successful new
25 anti-cancer therapeutic of the last three decades. Clearly, there is good justification for attempting to add to the list of mitotic inhibitors used to treat cancer. However, additional drugs that target tubulin or interfere with microtubule dynamics may be expected to have similar applicability and limitations as Taxol.

The task of the present invention therefore is to find new potential target proteins/genes for therapeutical drugs other than tubulin that are essential for completion of mitosis. These proteins/genes may provide novel targets to screen for new anti-neoplastic or cytotoxic anti-cancer agents.

5

Unfortunately, until now, the systematic identification of such target proteins/genes using genetic screening methods has been difficult in metazoans, and has relied heavily on the use of the unicellular yeast. Several major advances in the use of certain metazoan model organisms, particularly the nematode worm *Caenorhabditis elegans*, have now begun to
10 offer new ways of bridging this gap.

The above-mentioned task of the invention to find new potential target proteins/genes for therapeutical drugs other than tubulin involved in mitosis processes is solved by a screening assay in *C. elegans* based on 'genomic RNA mediated interference (RNAi)'
15 combined with a highly probative microscopic assay for documenting the first rounds of embryonic cell division (Sulston *et al.*, The embryonic cell lineage of the nematode *Caenorhabditis elegans*. *Dev. Biol.* 100, 64-119 (1983); Gönczy *et al.*, Dissection of cell division processes in the one cell stage *Caenorhabditis elegans* embryo by mutational analysis. *J Cell Biol* 144, 927-946 (1999)). With this combination of techniques a selected
20 gene and also a variety of selected genes can be functionally characterized with unprecedented speed and efficiency.

The nematode *C. elegans* exhibits an almost entirely translucent body throughout its development, thereby offering unparalleled microscopic access for exquisitely detailed
25 cytological documentation, even for the earliest steps of embryogenesis. This important feature, along with its short life cycle (3-5 days), its ease of cultivation, and its low maintenance costs, has helped make *C. elegans* arguably the best studied of all metazoans. Also, sequence data are now available for over 97% of the *C. elegans* genome (*C. elegans* Sequencing Consortium. Genome sequence of the nematode *C. elegans*: a platform for
30 investigating biology. *Science* 282, 2012-2018 (1998)). Thus, *C. elegans* has proven to be

an ideal organism for applying the new technique of RNA-mediated interference (RNAi). This technique consists in the targeted, sequence-specific inhibition of gene expression, as mediated by the introduction into an adult worm of double-stranded RNA (dsRNA) molecules corresponding to portions of the coding sequences of interest (Fire *et al.*, Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*. *Nature* 391, 806-811 (1998)). For the vast majority of *C. elegans* genes tested to date, this has been shown to yield a sequence-specific inhibition of the targeted gene's expression, accompanied by clearly detectable loss of function phenotypes in the treated worm's F1 progeny (and even in some cases, in the treated worm itself).

10

A large-scale RNAi technique-based screen was performed for 2,232 (that means 96%) of the predicted open reading frames on chromosome III of *C. elegans* which is described in detail in Gönczy *et al.*, "Functional genomic analysis of cell division in *C. elegans* using RNAi of genes on chromosome III" *Nature* 408, 331-336 (2000). For the performance of this large-scale screen double-stranded RNA corresponding to the individual open reading frames was produced and micro-injected into adult *C. elegans* hermaphrodites, and the resulting embryos were analysed 24 hours later using time-lapse DIC microscopy.

Besides others, the *C. elegans* genes H38K22.2 (Genbank/EMBL ID: AL024499, provided in SEQ ID NO. 1 - 3), C02F5.1 (Genbank/EMBL ID: L14745; , provided in SEQ ID NO. 4 and 5) and F10E9.8 (GenBank/EMBL ID: L10986; provided in SEQ ID NO. 6 and 7) gave rise to a phenotype detectable by the DIC-assay implying a functional role of these genes in metazoan cell division processes.

In at least one case (for H38K22.2) it had also been possible to identify a structurally and functionally homologous gene, a so-called orthologous gene, in another species, in particular *Homo sapiens*, namely the human orthologue RP42.

For the mouse orthologue of the RP42 gene it had merely been known that the gene shows a strongly developmentally regulated expression, particularly in proliferating neuroblasts from which neocortical neurons originate (Mas *et al.*, "Cloning and expression of a novel gene, RP42, mapping to an autism susceptibility locus on 6Q16" *Genomics* 1; 65 (1), 70-74 (2000)). The functional role of RP42 in cell division and proliferation processes that

30

- 5 -

makes it an excellent tool for the development or identification of drugs for diagnosis and/or therapy of proliferative diseases was not known so far.

With the essential function of said genes in cell division and proliferation known, these
5 newly identified target genes and their corresponding gene products, any homologues, orthologues and derivatives thereof represent excellent tools for use in the development and isolation of a wide range of therapeutics including anti-proliferative agents and in the development of methods for diagnosis and treatment of proliferative diseases.

10 Therefore, in a first aspect, the present invention relates to isolated nucleic acid molecules encoding a polypeptide functionally involved in cell division and proliferation or a fragment thereof and comprising a nucleic acid sequence selected from the group consisting of:

- 15 (a) the nucleic acid sequences presented in SEQ ID NO. 1 to 3, SEQ ID NO. 4 to 5, SEQ ID NO. 6 to 7, SEQ ID NO. 12 and fragments thereof and their complementary strands,
- (b) nucleic acid sequences encoding polypeptides that exhibit a sequence identity with SEQ ID NO. 8, SEQ ID NO. 9, SEQ ID NO. 10, SEQ ID NO. 11 or SEQ ID NO. 13 of at least 25 % over 100 residues and/or which are detectable in a
20 computer aided search using the blast sequence analysis programs with an e-value of at most 10^{-30} ,
- (c) nucleic acid sequences which are capable of hybridizing with the nucleic acid sequences of (a) or (b) under conditions of medium stringency,
- 25 (d) nucleic acid sequences which are degenerate as a result of the genetic code to any of the sequences defined in (a), (b) or (c).

- 6 -

The above mentioned fragments of the isolated nucleic acid molecules may comprise a at least 15 nucleotides and preferably at least 20 nucleotides.

Additionally the above mentioned isolated nucleic acid molecules may be single or double-stranded DNA-molecules as well as single- or double-stranded RNA-molecules.

5

a):

The nucleic acid sequences of those nucleic acid molecules encoding a polypeptide functionally involved in cell division and proliferation as mentioned in a) are provided in the sequence listing

- 10 as SEQ ID NO. 1 - 3 (*C. elegans* genes H38K22.2 (Genbank/EMBL ID: AL024499)),
as SEQ ID NO. 4 and 5 (*C. elegans* gene C02F5.1 (Genbank/EMBL ID: L14745)),
as SEQ ID NO. 6 and 7 (*C. elegans* gene F10E9.8 (GenBank/EMBL ID: L10986)) and
as SEQ ID NO. 12 (the human H38K22.2 orthologue, the RP42 protein (NCBI Accession No. AF292100)).
- 15 The corresponding deduced amino acid sequences of these target genes are disclosed in SEQ ID NO. 8 (for H38K22.2a), in SEQ ID NO. 9 (for H38K22.2b), in SEQ ID NO. 10 (for C02F5.1), in SEQ ID NO. 11 (for F10E9.8) and in SEQ ID NO. 13 (for RP42).

b):

- 20 Additionally, the present invention also comprises isolated nucleic acid molecules that are structurally and functionally homologous counterparts (particularly orthologues) of at least one of said target genes as disclosed in SEQ ID NO 1 to 7 or 12.

- Those homologous nucleic acid molecules may encode polypeptides that exhibit a sequence identity with SEQ ID NO. 8, SEQ ID NO. 9, SEQ ID NO. 10, SEQ ID NO. 11 or
25 SEQ ID NO. 13 of at least 25 % over 100 residues, preferably of at least 30 % over 100 residues, more preferably of at least 35 % over 100 residues and most preferably at least 40 % over 100 residues.

Fig. 5 shows that the aforementioned sequence identities are significant homologies that are appropriate to identify a polypeptide as an orthologue of the target proteins as depicted in SEQ ID NO. 8 -11, and 13. Fig. 5 shows a multiple sequence alignment of the H38K22.2a family on protein level generated with a BLAST sequence analysis program. In this alignment the two *C. elegans* splice variants H38K22.2a and H38K22.2b are compared to their corresponding orthologues in *Drosophila* (CG7427), in mouse (AAF04863) and in *Homo sapiens* (AAH09478). The statistics in Fig 5 for the alignments show that the sequence identity on protein level between the *C. elegans* clone H38K22.2a and its human orthologue (AAH09478) is 36 % over 299 residues. Similarly, the sequence identities between *C. elegans* clone H38K22.2b (the other splice variant) and its human orthologue is 36 % over 238 residues. It is obvious to anyone skilled in the art that these sequence homologies are significant homologies and that therefore the human clone with the accession No. AAH09478 is unambiguously identified as the human orthologue of the *C. elegans* clones H38K22.2a and H38K22.2b.

The invention also comprises isolated nucleic acid molecules that are detectable in a computer aided search using one of the BLAST sequence analysis programs with an e-value of at most 10^{-30} , preferably with an e-value of at most most 10^{-35} , more preferably with an e-value of at most most 10^{-40} .

Fig. 5 shows that the aforementioned e-values characterize significant sequence homologies that are appropriate to identify a polypeptide as an orthologue of the target proteins as depicted in SEQ ID NO. 8 -11, and 13.

The BLAST sequence analysis programs are programs used for sequence analysis that are publically available and known to anyone skilled in the art. When sequence alignments are done by a BLAST sequence analysis program, most of those programs calculate so called "e-values" to characterize the grade of homology between the compared sequences. Generally a small e-value characterizes a high sequence identity / homology, whereas larger e-values characterize lower sequence identities / homologies.

"Homology" means the degree of identity between two known sequences. As stated above, homologies, that means sequence identities, may suitably be determined by means of computer programs known in the art. The degree of homology required for the sequence variant will depend upon the intended use of the sequence. It is well within the capability

- 8 -

of a person skilled in the art to effect mutational, insertional and deletional mutations which are designed to improve the function of the sequence or otherwise provide a methodological advantage.

5 c):

The present invention further relates to isolated nucleic acid sequences or fragments thereof which are capable of hybridizing with the nucleic acid sequences of (a) or (b) under conditions of medium/high stringency.

10 The grade of sequence identity between a first and a second nucleic acid molecule can also be characterized by the capability of the first nucleic acid molecule to hybridize under certain conditions to a second nucleic acid molecule.

Suitable experimental conditions for determining whether a given DNA or RNA sequence "hybridizes" to a specified polynucleotide or oligonucleotide probe involve presoaking of the filter containing the DNA or RNA to examine for hybridization in 5 x SSC (sodium chloride/sodium citrate) buffer for 10 minutes, and prehybridization of the filter in a solution of 5 x SSC, 5 x Denhardt's solution, 0,5 % SDS and 100 mg/ml of denaturated sonicated salmon sperm DNA (Maniatis et al., 1989), followed by hybridization in the same solution containing a concentration of 10 ng/ml of a random primed (Feinberg, A.P. and Vogelstein, B. (1983), *Anal. Biochem.* 132:6-13), ³²P-dCTP-labeled (specific activity > 1 x 10⁹ cpm/μg) probe for 12 hours at approximately 45°C. The filter is then washed twice for 30 minutes in 2 x SSC, 0,5% SDS at at least 55°C (low stringency), at least 60°C (medium stringency), preferably at least 65°C (medium/high stringency), more preferably at least 70°C (high stringency) or most preferably at least 75°C (very high stringency). Molecules to which the probe hybridizes under the chosen conditions are detected using an x-ray film.

25

d):

The present invention further relates to isolated nucleic acid molecules or fragments thereof which are degenerate as a result of the genetic code to any of the sequences defined in (a), (b) or (c).

The application of automated gene synthesis provides an opportunity for generating sequence variants of the naturally occurring genes. It will be appreciated, for example, that polynucleotides coding for the same gene products can be generated by substituting synonymous codons for those represented in the naturally occurring polynucleotide sequences as identified herein. Such sequences will be referred to as “degenerate” to the naturally occurring sequences. In addition, polynucleotides coding for synthetic variants of the corresponding amino acid sequences can be generated which, for example, will result in one or more amino acids substitutions, deletions or additions. Also, nucleic acid molecules comprising one or more synthetic nucleotide derivatives (including morpholinos) which provide said nucleotide sequence with a desired feature, e.g. a reactive or detectable group, can be prepared. Synthetic derivatives with desirable properties may also be included in the corresponding polypeptides. All such derivatives and fragments of the above identified genes and gene products showing at least part of the biological activity of the naturally occurring sequences or which are still suitable to be used, for example, as probes for, e.g. identification of homologous genes or gene products, are included within the scope of the present invention.

Having herein provided the nucleotide sequences of various genes functionally involved in cell division and proliferation, it will be appreciated that automated techniques of gene synthesis and/or amplification may be used to isolate said nucleic acid molecules *in vitro*. Because of the length of some coding sequences, application of automated synthesis may require staged gene construction, in which regions of the gene up to about 300 nucleotides in length are synthesized individually and then ligated in correct succession for final assembly. Individually synthesized gene regions can be amplified prior to assembly, using polymerase chain reaction (PCR) technology. The technique of PCR amplification may also be used to directly generate all or part of the final genes/nucleic acid molecules. In this case, primers are synthesized which will be able to prime the PCR amplification of the final product, either in one piece or in several pieces that may be ligated together. For this purpose, either cDNA or genomic DNA may be used as the template for the PCR amplification. The cDNA template may be derived from commercially available or self-constructed cDNA libraries.

- 10 -

In a second aspect, the invention relates to nucleic acid probes comprising a nucleic acid sequence as previously characterized under (a) to (d) which may be a polynucleotide or an oligonucleotide comprising at least 15 nucleotides containing a detectable label.

These nucleic acid probes may be synthesized by use of DNA synthesizers according to standard procedures or, preferably for long sequences, by use of PCR technology with a
5 selected template sequence and selected primers. In the use of the nucleotide sequences as probes, the particular probe may be labeled with any suitable label known to those skilled in the art, including radioactive and non-radioactive labels. Typical radioactive labels include ^{32}P , ^{125}I , ^{35}S , or the like. A probe labeled with a radioactive isotope can be
10 constructed from a DNA template by a conventional nick translation reaction using a DNase and DNA polymerase. Non-radioactive labels include, for example, ligands such as biotin or thyroxin, or various luminescent or fluorescent compounds. The probe may also be labeled at both ends with different types of labels, for example with an isotopic label at one end and a biotin label at the other end. The labeled probe and sample can then be
15 combined in a hybridization buffer solution and held at an appropriate temperature until annealing occurs.

The invention also includes an assay kit comprising either an isolated nucleic acid molecule as defined above or a fragment thereof or a probe as defined above in a suitable
20 container.

Duplex formation and stability depend on substantial complementarity between the two strands of a hybrid and a certain degree of mismatch can be tolerated. Therefore, the nucleic acid molecules and probes of the present invention may include mutations (both single and multiple), deletions, insertions of the above identified sequences, and
25 combinations thereof, as long as said sequence variants still have substantial sequence homology to the original sequence which permits the formation of stable hybrids with the target nucleotide sequence of interest.

The above identified nucleic acid molecules and probes coding for polypeptides
30 functionally involved in cell division and proliferation or a part thereof will have a wide range of useful applications, including their use for identifying homologous, in particular orthologous, genes in the same or different species, their use in screening assays for

- 11 -

identification of interacting drugs that inhibit, stimulate or effect cell division or proliferation, their use for developing computational models, structural models or other models for evaluating drug binding and efficacy, and their diagnostic or therapeutic use for detection or treatment of diseases associated with anomalous and/or excessive cell division
5 or proliferation, in particular neoplastic diseases, including both solid tumors and hemopoietic cancers, or coronary restenosis. Exemplary neoplastic diseases include carcinomas, such as adenocarcinomas and melanomas; mesodermal tumors, such as neuroblastomas and retinoblastomas; sarcomas and various leukemias; and lymphomas. Of particular interest are tumors of the breast, ovaries, gastrointestinal tract, liver, lung,
10 thyroid glands, prostate gland, brain, pancreas, urinary tract, and salivary glands. Still more specific, tumors of the breast, ovaries, lung, colon, and lymphomas are contemplated.

In a third aspect, the present invention relates to the use of the above identified nucleic acid
15 molecules and probes for diagnostic purposes. This diagnostic use of the above identified nucleic acid molecules and probes may include, but is not limited to the quantitative detection of the expression of said target genes in biological probes (preferably, but not limited to cell extracts, body fluids, etc.), particularly by quantitative hybridization to the endogenous nucleic acid molecules comprising the above-characterized nucleic acid
20 sequences (particularly cDNA, RNA). An abnormal and/or excessive expression of said target genes involved in cell division may be diagnosed that way.

In a forth aspect, the present invention relates to the use of the above identified nucleic acid molecules, probes or their corresponding polypeptides for therapeutical purposes.
25

This therapeutical use of the above identified nucleic acid molecules, probes or their corresponding polypeptides may include, but is not limited to the use of said nucleic acid molecules and their corresponding polypeptides for direct or indirect inhibition of the expression of said target genes and/or for inhibition of the function of said target genes.
30 Particularly gene therapy vectors, e.g. viruses, or naked or encapsulated DNA or RNA (e.g. an antisense nucleotide sequence) with the above-identified sequences might be suitable

- 12 -

for the introduction into the body of a subject suffering from a proliferative disease or from a disease affecting cell division for therapeutical purposes.

A particularly preferred therapeutical use of the above identified nucleic acid molecules or
5 probes relates to their use in a therapeutical application of the RNAi technique, particularly in humans or in human cells.

Double-stranded RNA oligonucleotides effect silencing of the expression of gene(s) which are highly homologous to either of the RNA strands in the duplex. Recent discoveries reveal that this effect, called RNA interference (RNAi), that had been originally discovered
10 in *C. elegans*, can also be observed in cells, particularly in human cells. Therefore the invention further comprises the use of double-stranded RNA oligonucleotides with the above identified nucleotide sequences (as stated in a) to d)), preferably with a length of at least 15 nucleotides (nt), more preferably with a length of at least 20 nt, for therapeutical silencing of the expression of genes involved in cell division or proliferation in cells of
15 other species, particularly in human cells. This therapeutical use particularly applies to cells of an individual that suffers from a disease associated with anomalous and/or excessive cell division or proliferation, particularly a coronary restinosis or a neoplastic disease selected from the group consisting of lymphoma, lung cancer, colon cancer, ovarian cancer and breast cancer.

20

In a fifth aspect, the invention further comprises a nucleic acid construct or a recombinant vector having incorporated the nucleic acid molecules as defined in (a) to (d) or a fragment thereof.

“Nucleic acid construct” is defined herein as any nucleic acid molecule, either single- or
25 double-stranded, in which nucleic acid sequences are combined and juxtaposed in a manner which will not occur naturally. The vector may be any vector which can be conveniently subjected to recombinant DNA procedures. The choice of the vector will usually depend on the host cell into which it is to be introduced. The vector may be an extrachromosomal entity, the replication of which is independent of chromosomal
30 replication, e.g. a plasmid. Alternatively, the vector may be one which, when introduced into a host cell, is integrated into the host cell genome and replicated together with the chromosome(s) into which it has been integrated.

The vector is preferably an expression vector in which the nucleic acid molecule as defined in (a) to (d) or a fragment thereof is operably linked to heterologous or homologous control sequences. The term "control sequences" is defined herein to include all components
5 which are necessary or advantageous for expression of the coding nucleic acid sequence. Such control sequences include, but are not limited to, a promoter, a ribosome binding site, translation initiation and termination signals and, optionally, a repressor gene or various activator genes. Control sequences are referred to as "homologous" if they are naturally linked to the coding nucleic acid sequence of interest and referred to as "heterologous" if
10 this is not the case. The term "operably linked" indicates that the sequences are arranged so that they function in concert for their intended purpose, i.e. expression of the desired protein.

The promoter may be any DNA sequence which shows transcriptional activity in the host
15 cell of choice and may be derived from genes encoding proteins either homologous or heterologous to the host cell.

Examples of suitable promoters for directing the transcription in a bacterial host are, e.g., the phage Lambda P_R or P_L promoters, the *lac*, *trp* or *tac* promoters of *E. coli*, the promoter
20 of the *Bacillus subtilis* alkaline protease gene or the *Bacillus licheniformis* alpha-amylase gene.

Examples of suitable promoters for directing the transcription in mammalian cells are, e.g., the SV40 promoter (Subramani et al., *Mol. Cell. Biol.* 1 (1981), 854-864), the MT-1
25 (metallothionein gene) promoter (Palmiter et al., *Science* 222 (1983), 809-814) or the adenovirus 2 major late promoter.

Examples of suitable promoters for use in insect cells are, e.g., the polyhedrin promoter (Vasuvedan et al., *Febs. Lett* 311, (1992), 7-11), the *Autographa californica* polyhedrosis
30 basic protein promoter (EP 397 485), or the baculovirus immediate early gene 1 promoter (US 5,155,037, US 5,162,222).

- 14 -

Examples of suitable promoters for use in yeast cells include promoters from yeast glycolytic genes (Hitzeman et al., *J. Biol. Chem.* 255 (1980), 1203-12080; Alber and Kawasaki, *J. Mol. Appl. Gen.* 1 (1982), 419-434) and the ADH2-4c promoter (Russell et al., *Nature* 304 (1983), 652-654).

5

The coding sequence may, if necessary, be operably linked to a suitable terminator, such as the human growth hormone terminator (Palmiter et al., *Science* 222, 809-814 (1983)), or a polyadenylation sequence. Also, to permit secretion of the expressed protein, a signal sequence may precede the coding sequence.

10

Further, the vector may comprise a DNA sequence enabling the vector to replicate in the host cell in question. Examples of such sequences are the origins of replication of the plasmids pUC19, pACYC177, pUB110, pE194, pAMB1 and pIJ702. Another example of such a sequence (when the host cell is a mammalian cell) is the SV40 origin of replication.

15

When the host cell is a yeast cell, suitable sequences enabling the vector to replicate are the yeast plasmid 2 μ replication genes REP 1-3 and origin of replication.

The vector may also comprise a selectable marker, e.g. a gene coding for a product which complements a defect in the host cell, such as the gene coding for dihydrofolate reductase (DHFR) or a gene which confers resistance to a drug, e.g. ampicillin, kanamycin, tetracyclin, chloramphenicol, neomycin or hygromycin.

20

A number of vectors suitable for expression in prokaryotic or eukaryotic cells are known in the art and several of them are commercially available. Some commercially available mammalian expression vectors which may be suitable include, but are not limited to, pMC1neo (Stratagene), pXT1 (Stratagene), pSG5 (Stratagene), pcDNA1 (Invitrogen), EBO-pSV2-neo (ATCC 37593), pBPV-1(8-2) (ATCC 37110), pSV2-dhfr (ATCC 37146).

25

In a sixth aspect, the invention comprises host cells into which the nucleic acid construct or the recombinant vector is introduced. These host cells may be prokaryotic or eukaryotic, including, but not limited to, bacteria, fungal cells, including yeast and filamentous fungi,

30

- 15 -

mammalian cells, including, but not limited to, cell lines of human, bovine, porcine, monkey and rodent origin, and insect cells including, but not limited to, drosophila derived cell lines.

- 5 The selection of an appropriate host cell will be dependent on a number of factors recognized by the art. These include, e.g., compatibility with the chosen vector, toxicity of the (co)products, ease of recovery of the desired protein or polypeptide, expression characteristics, biosafety and costs.

Examples of suitable prokaryotic cells are gram positive bacteria such as *Bacillus subtilis*,
10 *Bacillus licheniformis*, *Bacillus brevis*, *Streptomyces lividans* etc. or gram negative bacteria such as *E. coli*.

The yeast host cell may be selected from a species of *Saccharomyces* or *Schizosaccharomyces*, e.g. *Saccharomyces cerevisiae*. Useful filamentous fungi may be selected from a species of *Aspergillus*, e.g. *Aspergillus oryzae* or *Aspergillus niger*.

- 15 Cell lines derived from mammalian species which may be suitable and which are commercially available include, but are not limited to, COS-1 (ATCC CRL 1650) COS-7 (ATCC CRL 1651), CHO-K1 (ATCC CCL 61), 3T3 (ATCCL 92), NIH/3T3 (ATCC CRL 1658), HeLa (ATCCL 2), and MRC-5 (ATCC CCL 171).

- 20 The recombinant vector may be introduced into the host cells according to any one of a number of techniques including, but not limited to, transformation, transfection, protoplast fusion, and electroporation.

The recombinant host cells are then cultivated in a suitable nutrient medium under
25 conditions permitting the expression of the protein of interest. The medium used to cultivate the cells may be any conventional medium suitable for growing the host cells, such as minimal or complex media containing appropriate supplements. Suitable media are available from commercial suppliers or may be prepared according to published recipes (e.g. in catalogues of the American Type Culture Collection).

Identification of the heterologous polypeptide expressing host cell clones may be done by several means, including, but not limited to, immunological reactivity with specific antibodies.

5 In a seventh aspect, the invention is related to a method for producing a polypeptide functionally involved in cell division and proliferation or a fragment thereof in a host cell comprising the steps

- (i) transferring the expression vector with an operably linked nucleic acid molecule as defined in (a) to (d) into a suitable host cell, and
- 10 (ii) cultivating the host cells of step (i) under conditions which will permit the expression of said polypeptide or fragment thereof and
- (iii) optionally, secretion of the expressed polypeptide into the culture medium.

In an eighth aspect, the invention comprises a polypeptide functionally involved in cell
15 division and proliferation or a fragment thereof comprising an amino acid sequence selected from the group consisting of:

- (a) the amino acid sequences depicted in SEQ ID NO. 8, 9, 10, 11 and 13 and fragments thereof,
- (b) amino acid sequences which exhibit a sequence identity with the sequences of
20 (a) of at least 25 % over 100 residues, preferably of at least 30 % over 100 residues, more preferably of at least 35 % over 100 residues and most preferably of at least 40 % over a 100 residues and/or which are detectable in a computer aided search using the BLAST sequence analysis programs with an e-value of at most 10^{-30} , preferably with an e-value of at most 10^{-35} and most
25 preferably with an e-value of at most 10^{-40} ,
- (c) amino acid sequences encoded by a nucleic acid molecule that is capable of hybridizing with the nucleic acid sequences of (a) or (b) or encoded by a nucleic acid molecule that is degenerate as a result of the genetic code to any of the sequences as defined in (a) or (b).

The heterologous polypeptide may also be a fusion polypeptide in which another polypeptide is fused at the N-terminus or the C-terminus of the polypeptide of interest or fragment thereof. A fused polypeptide is produced by fusing a nucleic acid sequence (or a portion thereof) encoding another polypeptide to a nucleic acid sequence (or a portion thereof) of the present invention. Techniques for producing fusion polypeptides are known
5 in the art and include ligating the coding sequences so that they are in frame and the expression of the fusion polypeptide is under control of the same promotor(s) and terminator.

10 Expression of the polypeptides of interest may also be performed using *in vitro* produced synthetic mRNA. Synthetic mRNA can be efficiently translated in various cell-free systems, including but not limited to, wheat germ extracts and reticulocyte extracts, as well as efficiently translated in cell based systems including, but not limited to, microinjection into frog oocytes, preferably *Xenopus* oocytes.

15 In a ninth aspect, the invention involves antibodies against the above identified polypeptides and against immunogenic fragments thereof. The term "antibody" as used herein includes both polyclonal and monoclonal antibodies, as well as fragments thereof, such as Fv, Fab and F(ab)₂ fragments that are capable of binding antigen or hapten. The
20 present invention also contemplates "humanized" hybrid antibodies wherein amino acid sequences of a non-human donor antibody exhibiting a desired antigen-specificity are combined with sequences of a human acceptor antibody. The donor sequences will usually include at least the antigen-binding amino acid residues of the donor but may comprise other structurally and/or functionally relevant amino acid residues of the donor antibody as
25 well. Such hybrids can be prepared by several methods well known in the art (see e.g. WO 89/09622; WO 94/11509; Couto, *Hybridoma* 13 (1994), 215-219; Presta, *Cancer Research* 57 (1997), 4593-4599). The antibodies of the present invention will have a wide range of useful applications, including their use for affinity purification of the corresponding immunogenic (poly)peptides, their use for the preparation of anti-idiotypic antibodies, as
30 well as their use as specific binding agents in various assays, e.g. diagnostic or drug-screening assays, or in a method for treatment of diseases associated with anomalous and/or excessive cell division or proliferation as exemplified above. Specifically, said

- 18 -

antibodies or suitable fragments thereof, particularly in humanized form, may be used as therapeutic agents in a method for treating cancer and other diseases associated with anomalous and/or excessive cell division or proliferation as exemplified above. Also, antibodies may be raised to the most characteristic parts of the above identified polypeptides and subsequently be used to identify structurally and/or functionally related polypeptides from other sources as well as mutations and derivatives of the above identified polypeptides.

To raise antibodies against the polypeptides of the present invention, there may be used as an immunogen either the intact polypeptide or an immunogenic fragment thereof, produced in a suitable host cell as described above or by standard peptide synthesis techniques.

Polyclonal antibodies are raised by immunizing animals, such as mice, rats, guinea pigs, rabbits, goats, sheep, horses etc., with an appropriate concentration of the polypeptide or peptide fragment of interest either with or without an immune adjuvant.

Acceptable immune adjuvants include, but are not limited to, Freund's complete adjuvant, Freund's incomplete adjuvant, alum-precipitate, water-in-oil-emulsion containing *Corynebacterium parvum* and tRNA.

In a typical immunization protocol each animal receives between about 0,1 µg and about 1000 µg of the immunogen at multiple sites either subcutaneously (SC), intraperitoneally (IP), intradermally or in any combination thereof in an initial immunization. The animals may or may not receive booster injections following the initial injection. Those animals receiving booster injections are generally given an equal amount of the immunogen in Freund's incomplete adjuvant by the same route at intervals of about three or four weeks until maximal titers are obtained. At about 7-14 days after each booster immunization or about weekly after a single immunization, the animals are bled, the serum collected, and aliquots are stored at about -20°C.

Monoclonal antibodies which are reactive with the polypeptide or peptide fragment of interest are prepared using basically the technique of Kohler and Milstein, Nature 256: 495-497 (1975). First, animals, e.g. Balb/c mice, are immunized using a protocol similar to that described above. Lymphocytes from antibody-positive animals, preferably

- 19 -

splenocytes, are obtained by removing spleens from immunized animals by standard procedures known in the art. Hybridoma cells are produced by mixing the splenocytes with an appropriate fusion partner, preferably myeloma cells, under conditions which will allow the formation of stable hybridomas. Fusion partners may include, but are not limited to:

5 mouse myelomas P3/NS1/Ag 4-1; MPC-11; S-194 and Sp 2/0. Fused hybridoma cells are selected by growth in a selection medium and are screened for antibody production. Positive hybridomas may be grown and injected into, e.g., pristane-primed Balb/c mice for ascites production. Ascites fluid is collected about 1-2 weeks after cell transfer and the monoclonal antibodies are purified by techniques known in the art. Alternatively, *in vitro*

10 production of monoclonal antibodies (mAb) is possible by cultivating the hybridomas in a suitable medium, e.g. DMEM with fetal calf serum, and recovering the mAb by techniques known in the art.

Recovered antibody can then be coupled covalently to a detectable label, such as a radiolabel, enzyme label, luminescent label, fluorescent label or the like, using linker

15 technology established for this purpose.

Antibody titers of ascites or hybridoma culture fluids are determined by various serological or immunological assays which include, but are not limited to, precipitation, passive agglutination, enzyme-linked immunosorbent antibody (ELISA) technique and radioimmunoassay techniques. Similar assays may be used to detect the presence of the

20 above identified polypeptides or fragments thereof in body fluids or tissue and cell extracts.

Assay kits for performing the various assays mentioned in the present application may comprise suitable isolated nucleic acid or amino acid sequences of the above identified genes or gene products, labelled or unlabelled, and/or specific ligands (e.g. antibodies)

25 thereto and auxiliary reagents as appropriate and known in the art. The assays may be liquid phase assays as well as solid phase assays (i.e. with one or more reagents immobilized on a support).

Unless otherwise specified, the manipulations of nucleic acids and polypeptides/-proteins can be performed using standard methods of molecular biology and immunology (see, e.g.

30 Maniatis et al. (1989), Molecular cloning: A laboratory manual, Cold Spring Harbor Lab., Cold Spring Harbor, NY; Ausubel, F.M. et al. (eds.) "Current protocols in Molecular

- 20 -

Biology". John Wiley and Sons, 1995; Tijssen, P., Practice and Theory of Enzyme Immunoassays, Elsevier Press, Amsterdam, Oxford, New York, 1985).

5 The invention further includes an assay kit comprising either the polypeptide as defined above or a fragment thereof or an antibody against said polypeptides as defined above or against immunogenic fragments thereof.

10 These recombinant polypeptides or fragments thereof as well as antibodies against those polypeptides or immunogenic fragments thereof will have a wide range of useful applications, including their use in screening assays for interacting drugs that inhibit, stimulate or effect the cell division or proliferation, their use for developing computational models, structural models or other models for evaluating drug binding and efficacy, and their use in a method for diagnosis or treatment of diseases associated with anomalous and/or excessive cell division or proliferation, in particular neoplastic diseases, including
15 both solid tumors and hemopoietic cancers, or coronary restenosis. Exemplary neoplastic diseases include carcinomas, such as adenocarcinomas and melanomas; mesodermal tumors, such as neuroblastomas and retinoblastomas; sarcomas and various leukemias; and lymphomas. Of particular interest are tumors of the breast, ovaries, gastrointestinal tract, liver, lung, thyroid glands, prostate gland, brain, pancreas, urinary tract, and salivary
20 glands. Still more specific, tumors of the breast, ovaries, lung, colon, and lymphomas are contemplated.

25 Therefore in a tenth aspect, the present invention explicitly includes the use of polypeptides as defined above or fragments thereof or of antibodies against said polypeptides or immunogenic fragments thereof in a screening assay for interacting drugs that inhibit, stimulate or effect the cell division or proliferation.

Such a screening assay for interacting drugs may particularly comprise, but is not limited to the following steps:

30

1. recombinant expression of said polypeptide or of an appropriate derivative thereof

2. isolation and optionally purification of the recombinantly expressed polypeptide or of its derivative, in particular by affinity chromatography
3. optionally labelling of the chemical compounds that are tested to interact with said polypeptide or its derivative and/or labelling of the recombinantly expressed polypeptide
4. immobilization of the recombinantly expressed polypeptide or of its derivative to a solid phase
5. binding of a potential interaction partner or a variety thereof to the immobilized polypeptide or its derivative
6. optionally one or more washing steps
7. detection and/or quantification of the interaction, in particular by monitoring the amount of label remaining associated with the solid phase over background levels.

Step 1 includes the recombinant expression of the above identified polypeptide or of its derivative from a suitable expression system, in particular from cell-free translation, bacterial expression, or baculovirus-based expression in insect cells.

Step 2 comprises the isolation and optionally the subsequent purification of said recombinantly expressed polypeptides with appropriate biochemical techniques that are familiar to a person skilled in the art.

Alternatively, these screening assays may also include the expression of derivatives of the above identified polypeptides which comprises the expression of said polypeptides as a fusion protein or as a modified protein, in particular as a GST-fusion protein or as a protein bearing a so called "tag"-sequence. These "tags"-sequences consist of short nucleotide sequences that are ligated 'in frame' either to the N- or to the C-terminal end of the coding region of said target gene. One of the most common tags that are used to label recombinantly expressed genes is the poly-Histidine-tag which encodes a homopolypeptide consisting merely of histidines. In this context the term "polypeptide" does not merely comprise polypeptides with the nucleic acid sequences of SEQ ID No. 1 bis 7, their naturally occurring homologues, preferably orthologues, more preferably human orthologues, in particular the RP42 gene (SEQ ID No. 12), but also derivatives of these polypeptides, in particular fusion proteins or polypeptides comprising a tag-sequence.

- 22 -

These polypeptides, particularly those labelled by an appropriate tag-sequence (for instance a His-tag) or by GST, may be purified by standard affinity chromatography protocols, in particular by using chromatography resins linked to anti-His-tag-antibodies or to anti-GST-antibodies which are both commercially available. Alternatively to the use of
5 anti-tag- or anti-GST-antibodies or other 'label-specific' antibodies the purification may also involve the use of antibodies against said polypeptides. Screening assays that involve a purification step of the recombinantly expressed target genes as described above (step 2) are preferred embodiments of this aspect of the invention.

In a third - optional - step the compounds tested for interaction may be labelled by
10 incorporation of radioactive isotopes or by reaction with luminescent or fluorescent compounds. Alternatively or additionally also the recombinantly expressed polypeptide may be labelled.

In a forth step the recombinantly expressed polypeptide is immobilized to a solid phase, particularly (but not limited) to a chromatography resin. The coupling to the solid phase is
15 thereby preferably established by the generation of covalent bonds.

In a fifth step a candidate chemical compound that might be a potential interaction partner of the said recombinant polypeptide or a complex variety thereof (particularly a drug library) is brought into contact with the immobilized polypeptide.

In a sixth - optional - step one or several washing steps may be performed. As a result just
20 compounds that strongly interact with the immobilized polypeptide remain bound to the solid (immobilized) phase.

In step 7 the interaction between the polypeptide and the specific compound is detected, in particular by monitoring the amount of label remaining associated with the solid phase over background levels.

25

Brief Description of the Drawings

Fig. 1 shows DIC microscopy images taken from time-lapse recording of the first two rounds of embryonic cell division in wild type *C. elegans*.

Fig. 2 shows DIC microscopy images taken from time-lapse recording of the first two
30 rounds of embryonic cell division in *C. elegans* F1 progeny from F0 parent treated with ds RNA "300C3" or "340G12" directed against gene H38K22.2.

Fig. 3 shows DIC microscopy images taken from time-lapse recording of the first two rounds of embryonic cell division in *C. elegans* F1 progeny from F0 parent treated with dsRNA "307C1" directed against gene C02F5.1.

Fig. 4 shows shows DIC microscopy images taken from time-lapse recording of the first two rounds of embryonic cell division in *C. elegans* F1 progeny from F0 parent treated with ds RNA "305A12" directed against gene F10E9.8.

Fig 5 shows a multiple sequence alignment of the H38K22.2a family. Herein, the amino acid sequences of the two *C. elegans* splice variants H38K22.2a and H38K22.2b are compared to the amino acid sequences of their orthologues in *Drosophila* (CG7427), in mouse (AAF04863) and in homo sapiens (AAH09478).

The "statistics" refer to values that characterize the grade of homology between the individual sequences, as the e-value, the sequence identities and the conservatively changed residues (positives).

15

Description of the sequence protocol:

- | | |
|--------------|--|
| SEQ ID NO. 1 | shows the unspliced DNA sequence common to both isoforms a and b of the <i>C. elegans</i> gene H38K22.2 (3104 bp). |
| SEQ ID NO. 2 | shows the spliced DNA sequence of the <i>C. elegans</i> gene H38K22.2a isoform (1011 bp). |
| SEQ ID NO. 3 | shows the spliced DNA sequence of the <i>C. elegans</i> gene H38K22.2b isoform (852 bp). |
| SEQ ID NO. 4 | shows the unspliced DNA sequence of the <i>C. elegans</i> gene C02F5.1 (3308 bp). |
| SEQ ID NO. 5 | shows the spliced DNA sequence of the <i>C. elegans</i> gene C02F5.1 (3033 bp). |
| SEQ ID NO. 6 | shows the unspliced DNA sequence of the <i>C. elegans</i> gene F10E9.8 |

- 24 -

- (7097 bp).
- SEQ ID NO. 7 shows the spliced DNA sequence of the *C. elegans* gene F10E9.8 (3624 bp).
- 5 SEQ ID NO. 8 shows the deduced amino acid sequence of the *C. elegans* gene H38K22.2a isoform (336 aa).
- SEQ ID NO. 9 shows the deduced amino acid sequence of the *C. elegans* gene H38K22.2b isoform (283 aa).
- SEQ ID NO. 10 shows the deduced amino acid sequence of the *C. elegans* gene C02F5.1 (1010 aa).
- 10 SEQ ID NO. 11 shows the deduced amino acid sequence of the *C. elegans* gene F10E9.8 (1207 aa).
- SEQ ID NO. 12 shows the cDNA sequence of a human orthologue of H38K22.2 (780 bp).
- SEQ ID NO. 13 shows the deduced amino acid sequence of a human orthologue of H38K22.2 (260 aa).
- 15

The following examples illustrate the present invention without, however, limiting the same thereto.

20

EXAMPLE 1: Generation of dsRNA molecules for RNAi experiments

First, oligonucleotide primer pair sequences were selected to amplify portions of the gene of interest's coding region using standard PCR techniques. Primer pairs were chosen to yield PCR products containing at least 500 bases of coding sequence, or a maximum of

25

- 25 -

coding bases for genes smaller than 500 bases. In order to permit the subsequent use of the PCR product as a template for *in vitro* RNA transcription reactions from both DNA strands, the T7 polymerase promoter sequence "TAATACGACTCACTATAGG" was added to the 5' end of forward primers, and the T3 polymerase promoter sequence
5 "AATTAACCCTCACTAAAGG" was added to the 5' end of reverse primers. The synthesis of oligonucleotide primers was completed by a commercial supplier (Sigma-Genosys, UK or MWG-Biotech, Germany).

PCR reactions were performed in a volume of 50 μ l, with Taq polymerase using 0.8 μ M primers and approximately 0.1 μ g of wild-type (N2 strain) genomic DNA template. The
10 PCR products were EtOH precipitated, washed with 70% EtOH and resuspended in 7.0 μ l TE. 1.0 μ l of the PCR reaction was pipetted into each of two fresh tubes for 5 μ l transcription reactions using T3 and T7 RNA polymerases. The separate T3 and T7 transcription reactions were performed according to the manufacturer's instructions (Ambion, Megascript kit), each diluted to 50 μ l with RNase-free water and then combined.
15 The mixed RNA was purified using RNeasy kits according to the manufacturer's instructions (Qiagen), and eluted into a total of 130 μ l of RNase-free H₂O. 50 μ l of this was mixed with 10 μ l 6X injection buffer (40 mM KPO₄ pH 7.5, 6 mM potassium citrate, pH 7.5, 4% PEG 6000). The RNA was annealed by heating at 68°C for 10 min, and at 37°C for 30 min. Concentration of the final dsRNAs were measured to be in the range of 0.1-0.3
20 μ g/ μ l. The products of the PCR reaction, of the T3 and T7 transcription reactions, as well as the dsRNA species were run on 1% agarose gels to be examined for quality control purposes. Success of double stranding was assessed by scoring shift in gel mobility with respect to single stranded RNA, when run on non-denaturing gels.

25

EXAMPLE 2: Injections of dsRNA and phenotypic assays

dsRNAs were injected bilaterally into the syncitial portion of both gonads of wild-type (N2 strain) young adult hermaphrodites, and the animals incubated at 20°C for 24 hrs.
30 Embryos were then dissected out from the injected animals and analyzed by time-lapse

- 26 -

differential interference contrast videomicroscopy for potential defects in cell division processes, capturing 1 image every 5 seconds, as previously described (Gönczy *et al.*, Dissection of cell division processes in the one cell stage *Caenorhabditis elegans* embryo by mutational analysis. *J Cell Biol* 144, 927-946 (1999)). For each experiment, embryos
5 from at least 3 different injected worms were filmed in this manner, from shortly after fertilization until the four cell stage. Embryos from 2 additional injected worms were also recorded via still images, thus yielding phenotypic documentation for at least 5 injected worms in each experiment.

In some cases, embryos exhibited acute sensitivity to osmotic changes, as evidenced by
10 their loss of structural integrity during the dissection of the injected animals. In order to overcome this limitation, injected animals were not dissected, but rather, anaesthetized for 10 min in M9 medium containing 0.1% tricaine and 0.01% tetramisole, and mounted intact on an agarose pad to observe the F1 embryogenesis *in utero* (Kirby *et al.*, *Dev. Biol.* 142, 203-215 (1990)). The resolution achieved by viewing through the body wall does not equal
15 that achieved by observing dissected embryos, and only limited phenotypic analysis was conducted in these cases.

Three injected animals were also transferred to a fresh plate 24 hrs after injection of dsRNA, and left at 20°C. Two days later, the plate was checked with a stereomicroscope (20-40x total magnification) for the presence of F1 larvae (L2's-L4's), as well as their
20 developmental stage. Two days after that, the plate was inspected again for the presence of F1 adults, as well as their overall body morphology and the presence of F2 progeny.

EXAMPLE 3: Characterization of the *C. elegans* gene H38K22.2

25 Two dsRNAs, "300C3" and "340G12", were designed and used to specifically silence the expression of the *C. elegans* gene H38K22.2 by RNAi, thereby testing its functional involvement in the first 2 rounds of embryonic cell division in this metazoan species. The dsRNAs were synthesized *in vitro* from PCR-amplified wild type genomic DNA fragments
30 of the H38K22.2 gene. For the PCR, two sets of primer pairs were used:

- 27 -

"TCAATCAGTATGTCGACCC" with "GGAAGAAATTGGGGAAACA" as forward and reverse primers, respectively, to generate dsRNA "300C3", and "ATCGAGCGCCTCTTCAATC" with "TGGTGTCTCCATTGCTGA" as forward and reverse primers, respectively, to generate dsRNA "340G12". The dsRNAs were purified, and injected into adult hermaphrodite worms. The phenotypic consequences of the RNAi treatment were documented 24 hours later in the F1 progeny of injected worms, using time-lapse differential interference contrast (DIC) microscopy. Embryo recordings started ~20 minutes after fertilisation, while the female pronucleus is completing its meiotic divisions, until the 4 cell stage, ~30 minutes later.

10 In the F1 progeny of control worms that were either not injected, or injected with irrelevant dsRNA, the cellular events of the first two rounds of embryonic cell division were found to exhibit very limited variability, as observed by DIC microscopy. All processes that were examined and scored for the possibility of phenotypic deviations are listed and illustrated in Figure 1. Briefly, the antero-posterior polarity of the embryo is initially determined by the position of the male pronucleus at the cortex, shortly after entry into the egg (right arrow in Fig. 1a). This is accompanied by a clear, coordinated flow of yolk granules through the central portion of the cytoplasm along the embryo's longitudinal axis towards the male pronucleus, and a concomitant series of cortical waves or ruffles progressing towards the anterior of the embryo (left side in Fig.1). Shortly thereafter, the male and female pronuclei undergo highly patterned migrations (right and left arrows respectively, in Fig. 1a,b) resulting in their meeting within the posterior half of the embryo (Fig. 1c), followed by a centration and rotation (Fig. 1d) of the pronuclear pair and associated centrosomes (arrowheads in Fig. 1b-d) to set up the future mitotic spindle along the embryo's longitudinal axis. After synchronous breakdown of the pronuclear envelopes, the clearly bipolar mitotic spindle is initially short (Fig. 1e), but then elongates while exhibiting clear lateral "rocking" movements of the posterior pole (Fig. 1f-h). These movements are accompanied by a slight posterior displacement of the posterior spindle pole, while the anterior spindle pole remains approximately stationary. This then results in an asymmetric positioning of the spindle during anaphase and telophase, thereby yielding an asymmetric placement of the cytokinetic furrow (arrowheads in Fig. 1i,j), and generating unequally-sized daughter cells: a smaller posterior P1 blastomere (right cell in Fig. 1k-o), and larger anterior AB blastomere (left cell in Fig. 1k-n). While the AB nucleus

- 28 -

then migrates directly to the center of the AB cell (left arrow in Fig. 1k-l), the P1 nucleus typically migrates further towards the posterior of that cell (right arrow in Fig. 1k-l), before undergoing a pronounced 90° rotation while re-migrating to the anterior P1 cortex with one of its duplicated centrosomes leading (arrowheads in Fig. 1m). This insures that the P1
5 blastomere then divides along the embryo's longitudinal axis, perpendicular to that of the AB blastomere (Fig. 1n, arrowheads indicate centrosomes). These two divisions occur asynchronously, with P1 lagging 2-3 minutes behind AB (Fig. 1 n-p).

In the F1 embryos of worms injected with dsRNAs "300C3" or "340G12", the following highly reproducible phenotypes are observed (Fig. 2). First, although the dynamics of
10 female pronuclear migration appear normal in all cases, its initiation is often somewhat delayed. Meeting and apposition of the two pronuclei also typically exhibits defects in that the female pronucleus gets captured by only one of the two centrosomes associated with the male pronucleus (compare Fig. 2a-c with Fig. 1a-c). Although this defect is usually corrected before pronuclear envelope breakdown is completed, subsequent positioning of
15 the mitotic spindle within the embryo often appears defective. Weak manifestation of this phenotype appears as a lack of rocking of the posterior spindle pole during anaphase, while more severe cases show a notable drift of the entire spindle towards the posterior or lateral cortex, reaching the cortex itself and losing its longitudinal alignment completely. In the latter cases, the strongly aberrant spindle position gives rise to inappropriate specification
20 of cleavage furrow formation, leading to anomalous cytokinesis. Even in cases where spindle position appears relatively normal, positioning of the daughter Nucleus-Centrosomes-Complexes (NCCs) typically appears abnormal as soon as anaphase ends and the cleavage furrow ingresses. This is often particularly visible in the AB blastomere, where the NCC, instead of moving directly to the centre of the cell starting at telophase,
25 first migrates anteriorly in close proximity to the lateral cortex before eventually centering (Fig. 2a-k). This defect is usually accompanied by an apparent absence of interzonal spindle microtubules at telophase and a notable bifurcation or forking of the cytokinetic cleavage furrow (arrows in Fig. 2 g), leading to aberrantly-sized daughter blastomeres or even failure of cytokinesis by complete regression of the furrow (Fig. 2g-m). Nuclear
30 migration and positioning of the P1 nucleus is also aberrant in most cases, resulting in a significant delay - or in some cases, a complete failure - in achieving its expected 90° rotation and association with the anterior cortex. Division of the P1 blastomere is often

significantly delayed in such embryos. Finally, defects in female meiotic divisions are also occasionally observed, as evidenced by the presence of multiple female pronuclei, indicating a failure to successfully extrude one or both polar bodies, which could come from cytokinetic defects similar to those noted above.

5 All observed phenotypes indicate a requirement for H38K22.2 gene function in the microtubule-dependent cellular positioning of NCCs and spindles during mitosis, and possibly meiosis. Since this function is essential to cell cycle progression and cell division throughout metazoans, this gene and any homologues and derivatives thereof represent excellent tools for use in the development of a wide range of therapeutics including anti-
10 proliferative agents. Analysis of the H38K22.2 gene sequence reveals clear orthologues in human (NCBI Accession # AAH09478), mouse (NCBI Accession # AAF04863) and *Drosophila* (NCBI Accession # CG7427) (see Fig. 5), all of which have had no known functions ascribed to them until now. Based on their extremely high level of sequence conservation at the protein level, it can be concluded that all of these genes most likely
15 encode proteins with equivalent functions in each of their respective species. The 336 residue protein encoded by the H38K22.2 gene isoform "a" exhibits no known structural motifs or consensus domains, according to either SMART or CDD analyses.

20 **EXAMPLE 4: Characterization of the *C. elegans* gene C02F5.1**

A dsRNA, "307C1", was designed and used to specifically silence the expression of the *C. elegans* gene C02F5.1 by RNAi, thereby testing its functional involvement in the first 2 rounds of embryonic cell division in this metazoan species. The dsRNA was synthesized *in*
25 *vitro* from a PCR-amplified wild type genomic DNA fragment of the C02F5.1 gene. For the PCR, oligonucleotides with sequences "ATCTGAAGATCCGTCCACT" and "ATGCACAATGGGTATTTTT" were used as forward and reverse primers, respectively, to generate dsRNA "307C1" which was purified, and injected into adult hermaphrodite worms. The phenotypic consequences of the RNAi treatment were documented 24 hours
30 later in the F1 progeny of injected worms, using time-lapse differential interference contrast (DIC) microscopy. Embryo recordings started ~20 minutes after fertilisation,

- 30 -

while the female pronucleus is completing its meiotic divisions, until the 4 cell stage, ~30 minutes later.

In the F1 progeny of control worms that were either not injected, or injected with irrelevant dsRNA, the cellular events of the first two rounds of embryonic cell division were found to exhibit very limited variability, as observed by DIC microscopy. All processes that were examined and scored for the possibility of phenotypic deviations are listed and illustrated in Figure 1.

F1 embryos from parent worms injected with dsRNA "307C1" are consistently found to exhibit the following phenotypes (Fig. 3). First, all cellular processes that are scorable by DIC microscopy until entry into mitosis are typically indistinguishable from the wild type pattern. These include egg shape and size, yolk granule size and density, yolk granule flows and cortical ruffling, pseudo-cleavage furrow formation and positioning, pronuclear appearance (arrows in Fig. 3a) and migration (Fig. 3a,b), as well as centration and rotation of pronuclei (Fig. 3b,c) and associated pair of centrosomes (arrowheads in Fig. 3b,c). Formation and positioning of the bipolar mitotic spindle also take place normally, but the spindle is most often thinner and less rigid than in wild type, exhibiting aberrant lateral bending during its rocking and elongation at anaphase (Fig. 3f-i). After completion of cytokinesis, which appears normal, the reforming daughter nuclei are typically tear-shaped, and remain close to the newly-formed cortex for a prolonged period (Fig. 3a and k). Consistent with the tear shape, the two nuclei remain often physically connected by anomalous chromatin bridges and karyomeres are also typically seen (asterisks in Fig. 3k and l). This phenotype subsequently results in embryonic lethality in all cases.

The absence of defects in pronuclear migration and assembly of the bipolar spindle argue against a role for this gene in more general microtubule functions. The observed defects are consistent with a failure in mitotic chromosome segregation, most likely in the separation of sister chromatids, resulting in the formation of chromatin bridges, which then persist at telophase. The present data therefore indicate an essential requirement for C02F5.1 gene function in mitotic chromosome segregation. Since this function is essential to cell cycle progression and cell division throughout metazoans, this gene and any

homologues and derivatives thereof represent excellent tools for use in the development of a wide range of therapeutics including anti-proliferative agents.

Analysis of the C02F5.1 sequence reveals that the encoded 1010 residue protein contains regions predicted to form coiled coil structures, i.e. likely protein-protein interaction domains. Sequence homology analyses using the BLASTp program presently reveal no clearly orthologous sequences in other organisms. However, considering the essential and highly conserved nature of the cellular process in question, functional orthologues of this gene/protein are extremely likely to exist in all metazoans, possibly in all eukaryotes, and will be identified using for example the methodology as outlined in EXAMPLE 6.

10

EXAMPLE 5: Characterization of the *C. elegans* gene F10E9.8

15

Two dsRNAs, "305A12" and "341G5", were designed and used to specifically silence the expression of the *C. elegans* gene F10E9.8 by RNAi, thereby testing its functional involvement in the first 2 rounds of embryonic cell division in this metazoan species. The dsRNAs were synthesized *in vitro* from PCR-amplified wild type genomic DNA fragments of the F10E9.8 gene. For PCR, two sets of primer pairs were used: "TTCGTCTCGAACACGTATATCCT" with "GAAAGAAGATGAATCAGGCATTG" as forward and reverse primers, respectively, to generate dsRNA "305A12", and "CTGCAAAAATTATGACTGTGTGCG" with "AGCATTCAGATTTGGTTGTCC" as forward and reverse primers, respectively, to generate dsRNA "341G5". The dsRNA was purified, and injected into adult hermaphrodite worms. The phenotypic consequences of the RNAi treatment were documented 24 hours later in the F1 progeny of injected worms, using time-lapse differential interference contrast (DIC) microscopy. Embryo recordings started ~20 minutes after fertilisation, while the female pronucleus is completing its meiotic divisions, until the 4 cell stage, ~30 minutes later.

25

30 In the F1 progeny of control worms that were either not injected, or injected with irrelevant dsRNA, the cellular events of the first two rounds of embryonic cell division were found to

exhibit very limited variability, as observed by DIC microscopy. All processes that were examined and scored for the possibility of phenotypic deviations are listed and illustrated in Figure 1.

In the F1 embryos of worms injected with dsRNAs "305A12" or "341G5", the following
5 highly reproducible phenotypes are observed (Fig. 4). First, all cellular processes that are scorable by DIC microscopy until the 2-cell stage are typically indistinguishable from the wild type pattern. These include egg shape and size, yolk granule size and density, yolk granule flows and cortical ruffling, pseudo-cleavage furrow formation and positioning, pronuclear appearance (arrows in Fig. 4a) and migration (Fig. 4a,b), as well as centration
10 and rotation of pronuclei (Fig. 4b,c) and associated pair of centrosomes (arrowheads in Fig. 4b,c). The first round of division also occurs without any detectable deviations from wild type (Fig. 4d-h). It should particularly be noted that no defects are observed with respect to size, number or positioning of centrosomes or spindle poles in the single cell embryo (note arrowheads in Fig. 4b-f). In the two-cell stage embryo, however, although nuclear
15 positioning also remains equivalent to wild type, an apparent failure in centrosome duplication is consistently observed in one of the two blastomeres and sometimes in both. A single perinuclear centrosomal region, as seen by its exclusion of yolk granules (black arrowhead in Fig. 4h-j), is typically observed instead of the two normally seen both in wild type embryos and in the unaffected blastomere (white arrowheads in Fig. 4i,j). Despite the
20 apparent failure in centrosome duplication, microtubule-dependent processes continue normally, as illustrated by the successful anterior migration of the P1 nucleus, with its single centrosomal region leading (black arrowhead in Fig. 4h-j). Upon entering mitosis, as scored by nuclear envelope breakdown, the defective blastomere then fails to generate a bipolar spindle, forming instead a monopolar array of microtubules (dashed circle in Fig.
25 4k), as evidenced by the radial alignments of yolk granules in that region. Cytokinesis fails to occur in that blastomere, resulting in reformation of multiple, irregularly sized nuclei, known as karyomeres (arrows in Fig. 4m,n). In contrast, all aspects of cell division occur normally in the neighboring blastomere, resulting in normal daughter cells, each containing a single equal-sized nucleus (arrows in Fig. 4l).

30 The complete failure in bipolar spindle formation, accompanied by the presence of a single centrosomal region instead of two in the affected two-cell stage blastomere, clearly

- 33 -

indicates a requirement for F10E9.8 gene function in the complex process of mitotic spindle assembly. However, the lack of detectable defects in other microtubule-dependent processes including pronuclear migration and spindle function in the single-cell embryo effectively rules out a general microtubule-related function. In view of the maternal nature
5 of the RNAi effect and the fact that the egg inherits its first centrosome paternally, the successful generation of a bipolar spindle in the single-cell embryo further suggests that F10E9.8 function may, in fact, be required for some aspect of centrosome duplication or separation.

Indeed, since sperm development is fully completed within the parent before initiation of
10 the RNAi treatment, it remains unaffected by the injected dsRNA. This results in the donation of an intact "wild type" centrosome from the sperm to the egg at fertilisation. After fertilisation, this already bipartite centrosome (i.e. containing two "replication units", as evidenced by the presence of two centrioles) undergoes one round of duplication, as observed in other systems by the budding of a new centriole barrel from each existing
15 centriole. This is followed by a physical separation of the two centriole pairs and associated pericentriolar material. This process is not dependent on the prior duplication event, and is solely needed to insure the successful formation of the bipolar spindle to be used in the first round of embryonic cell division. It therefore appears that F10E9.8 function is most likely not required for this process.

20 5. If the first duplication round fails, however, bipolar spindle formation is expected to fail during the second round of division, as seen here. Interestingly, the fact that this failure often occurs only in one of the two blastomeres suggests that in these cases only one of the original centrosome's two "replication units" actually failed in its first round of duplication at the single-cell stage. This observation is consistent with findings from other eukaryotes
25 indicating that one of the two replication units contained within the sperm's centrosome actually comes into the egg already fully equipped for one duplication round, while the other must rely on cytoplasmic factors within the egg to permit its own duplication (Sluder, G., Hinchcliffe EH. Control of centrosome reproduction: the right number at the right time. *Biol. Cell.* 91, 413-27 (1999)).

- 34 -

The present findings therefore suggest that the requirement for F10E9.8 function in mitotic spindle assembly most likely results from this gene's essential role in the process of centrosome duplication.

5 Since the process of spindle assembly is essential to cell cycle progression and cell division throughout metazoans, this gene and any homologues and derivatives thereof represent excellent tools for use in the development of a wide range of therapeutics including anti-proliferative agents. Analysis of the F10E9.8 sequence reveals that the encoded 1207
10 residue protein contains one large region predicted to form coiled coil structures, i.e. likely protein-protein interaction domains, and four predicted transmembrane domains. Sequence homology analyses using the BLASTp program presently reveal no clearly orthologous sequences in other organisms. However, considering the essential and highly conserved nature of the cellular process in question, functional orthologues of this gene/protein are extremely likely to exist in all metazoans, possibly all eukaryotes, and will be identified
15 using for example the following methodology.

EXAMPLE 6: Protocol for identifying functional orthologues in other species

20 The present invention describes genes identified as having essential functions in cell division in the model organism *C. elegans*. The basis for performing research in model organisms is that the newly discovered functions for the genes in *C. elegans* will be conserved in other species including humans. Cell division is highly conserved during evolution and therefore the approach of discovering a gene function in *C. elegans* and
25 using the information to characterise or assign functions for the human orthologue is well justified. There are two themes of conservation of genes during evolution. A gene sequence may be conserved. This means that the DNA nucleotide sequence of the gene is very similar in different species, which in turn suggests that the function of the gene is the same in the different species. As is known to any person skilled in the art, a sequence
30 identity or homology above a particular level defines that two genes in different species code for the same gene product and gene function. Homologous genes are typically

- 35 -

identified by performing blast analysis with appropriate software, or by other approaches. For a blast search, an e-value of 10^{-30} will extract the significant homologous sequences. Further phylogenetic analysis can be performed to identify which of the extracted sequences are the orthologues.

- 5 Therefore the following example for identification of orthologues can be presented. A blast search is performed using the blast sequence analysis programs and an e-value of 10^{-3} . An alternative parameter can be the percentage of sequence identity. Over 100 residues, a sequence identity of 30% defines a homologous gene. After the blast search is completed, multiple sequence alignment is performed using appropriate software (for example,
10 CLUSTALW) and a neighbour joining phylogenetic tree is generated. Any person skilled in the art can identify the human orthologue from a phylogenetic tree. Essentially, the human sequence that is separated on the tree by a single speciation event or most closely related on the tree is likely to be an orthologue.

- The second theme of conservation is that the gene function can be conserved with greater
15 divergence of sequence. In the present invention this theme of conservation is not defined. However, if other genes are discovered to have functions that result in the gene product being identified as the same gene product as those claimed in the present invention then the present claims also apply to such genes.

Claims

1. An isolated nucleic acid molecule encoding a polypeptide functionally involved in cell
5 division and proliferation or a fragment thereof and comprising a nucleic acid
sequence selected from the group consisting of:
 - (a) the nucleic acid sequences presented in SEQ ID NO. 1 to 3, SEQ ID NO. 4 to 5,
SEQ ID NO. 6 to 7, SEQ ID NO. 12 and fragments thereof and their
complementary strands,
 - 10 (b) nucleic acid sequences encoding polypeptides that exhibit a sequence identity with
SEQ ID NO. 8, SEQ ID NO. 9, SEQ ID NO. 10, SEQ ID NO. 11 or SEQ ID NO.
13 of at least 25 % over 100 residues and/or which are detectable in a computer
aided search using the blast sequence analysis programs with an e-value of at most
 10^{-30} ,
 - 15 (c) nucleic acid sequences which are capable of hybridizing with the nucleic acid
sequences of (a) or (b) under conditions of medium/high stringency,
 - (d) nucleic acid sequences which are degenerate as a result of the genetic code to any
of the sequences defined in (a), (b) or (c).
2. A nucleic acid probe comprising a nucleic acid sequence as defined in claim 1 which
20 may be a polynucleotide or an oligonucleotide comprising at least 15 nucleotides
containing a detectable label.
3. A recombinant vector or nucleic acid construct having incorporated therein the
isolated nucleic acid molecule of claim 1 or a fragment thereof.
- 25 4. The vector of claim 3 which is an expression vector.
5. A host cell which has been genetically engineered to incorporate therein the isolated
nucleic acid molecule of claim 1 or the recombinant vector or nucleic acid construct of
claim 3.
- 30 6. The host cell of claim 5 having incorporated therein the expression vector of claim 4.

7. An assay kit comprising the isolated nucleic acid molecule or a fragment thereof of claim 1 or the probe of claim 2 in a suitable container.
- 5 8. A method for producing a polypeptide functionally involved in cell division and proliferation or a fragment thereof in a host cell comprising the steps
- (a) transferring the expression vector of claim 4 into a suitable host cell, and
 - (b) cultivating the host cells of step (a) under conditions which will permit the expression of said polypeptide or fragment thereof and
 - 10 (c) optionally, secretion of the expressed polypeptide into the culture medium.
9. Use of a probe as defined in claim 2 to isolate orthologues of genes comprising the nucleic acid sequences as disclosed in SEQ ID NO. 1 to 3, SEQ ID NO. 4 to 5, SEQ ID NO. 6 to 7, SEQ ID NO. 11.
- 15 10. Use of the isolated nucleic acid molecule or a fragment thereof as defined in claim 1 for producing a polypeptide functionally involved in cell division and proliferation or a fragment thereof.
- 20 11. Use of a nucleic acid molecule or a fragment thereof as defined in claim 1 or of the probe of claim 2 in a screening assay for interacting drugs that inhibit, stimulate or effect the cell division or proliferation.
- 25 12. Use of a nucleic acid molecule as defined in claim 1 or of the probe of claim 2 in a method for diagnosis or treatment of diseases associated with anormalous and/or excessive cell division or proliferation.
- 30 13. The use of claim 12 wherein the disease is a coronary restenosis or a neoplastic disease selected from the group consisting of lymphoma, lung cancer, colon cancer, ovarian cancer and breast cancer.

14. A polypeptide functionally involved in cell division and proliferation or a fragment thereof comprising an amino acid sequence selected from the group consisting of:
- (a) the amino acid sequences depicted in SEQ ID NO. 8, 9, 10, 11 and 13 and fragments thereof,
- (b) amino acid sequences which exhibit a sequence identity with the sequences of (a) of at least 25 % over 100 residues and/or which are detectable in a computer aided search using the BLAST sequence analysis programs with an e-value of at most 10^{-30} ,
- (c) amino acid sequences encoded by any of the nucleic acid sequences (c) – (d) as defined in claim 1.
15. A fusion protein comprising the polypeptide or fragment thereof of claim 14.
16. An antibody or a fragment thereof capable of specifically binding with the polypeptide of claim 14 or with an immunogenic part thereof.
17. A humanized antibody capable of specifically binding with the polypeptide of claim 14 or with an immunogenic part thereof.
18. An assay kit comprising the polypeptide as claimed in claim 14, the fusion protein as claimed in claim 15, or the antibodies as claimed in claims 16 and/or 17 in a suitable container.
19. Use of the polypeptide of claim 14, of the fusion protein of claim 15, or of the antibodies of claims 16 or 17 in a screening assay for interacting drugs that inhibit, stimulate or effect the cell division or proliferation.
20. The use of a polypeptide or of an antibody as claimed in claim 19 wherein the screening assay for interacting drugs comprises the following steps:

1. recombinant expression of said polypeptide in a host cell
 2. isolation and optionally purification of the recombinantly expressed polypeptide of step 1
 3. optionally labelling of the drugs that are tested to interact with said polypeptide
5 and/or labelling of the recombinantly expressed polypeptide
 4. immobilization of the recombinantly expressed polypeptide to a solid phase
 5. binding of a potential interaction partner or a variety thereof to the polypeptide
 6. optionally one or more washing steps
 7. detection and/or quantification of the interaction, in particular by monitoring
10 the amount of label remaining associated with the solid phase over background levels.
-
21. Use of the polypeptide of claim 14, of an amino acid sequence as defined in claim 14
or of the antibodies of claims 16 or 17 in a method for diagnosis or treatment of
15 diseases associated with anomalous and/or excessive cell division or proliferation.
-
22. The use of claim 20 wherein the disease is a coronary restenosis or a neoplastic disease
selected from the group consisting of lymphoma, lung cancer, colon cancer, ovarian
cancer and breast cancer.
20
-
22. Use of the nucleic acid sequences as defined in claim 1 or the amino acid sequences as
defined in claim 14 for developing computational models, structural models or other
models for evaluating drug binding and efficacy.

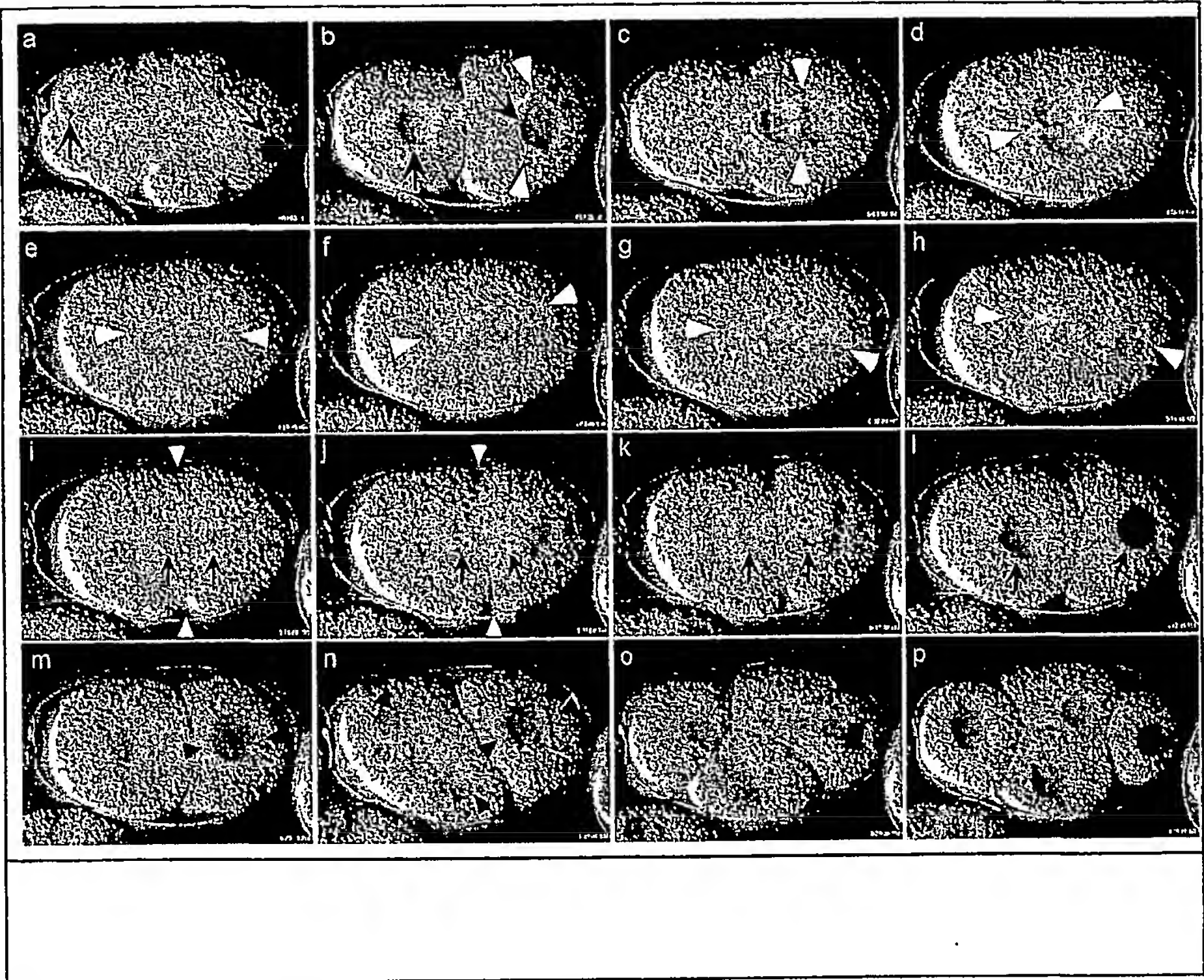


FIG. 1

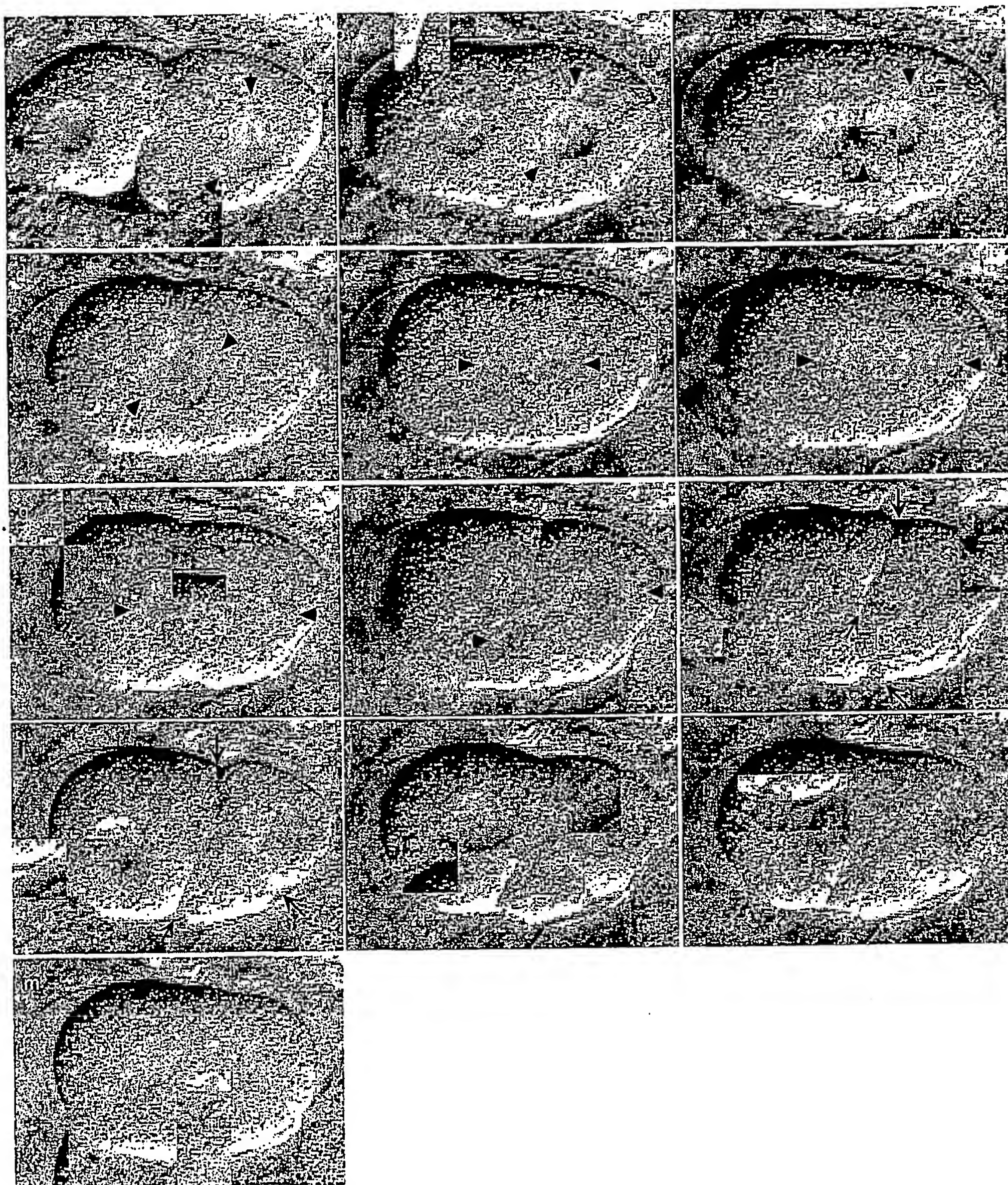


FIG. 2

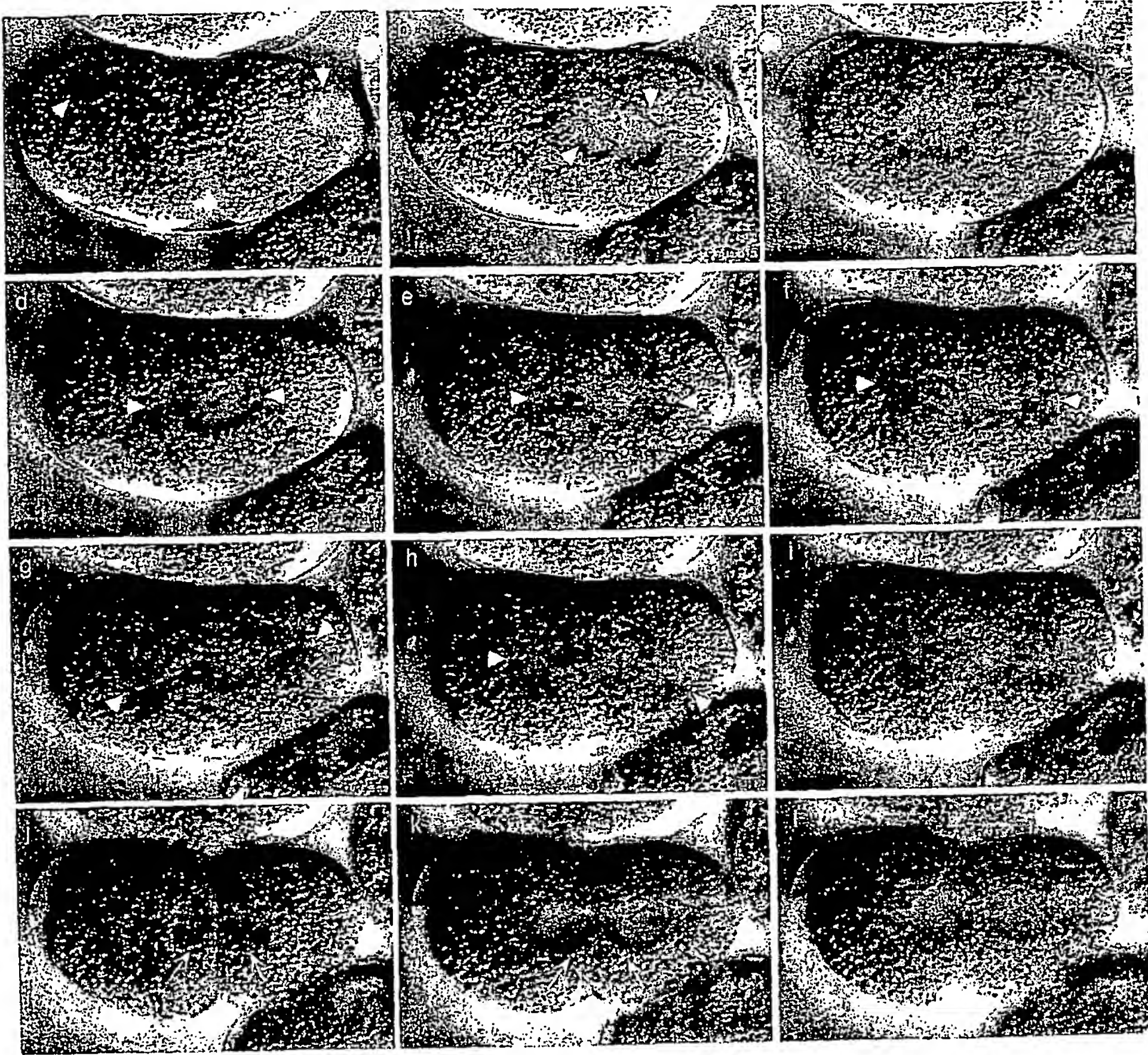


FIG. 3

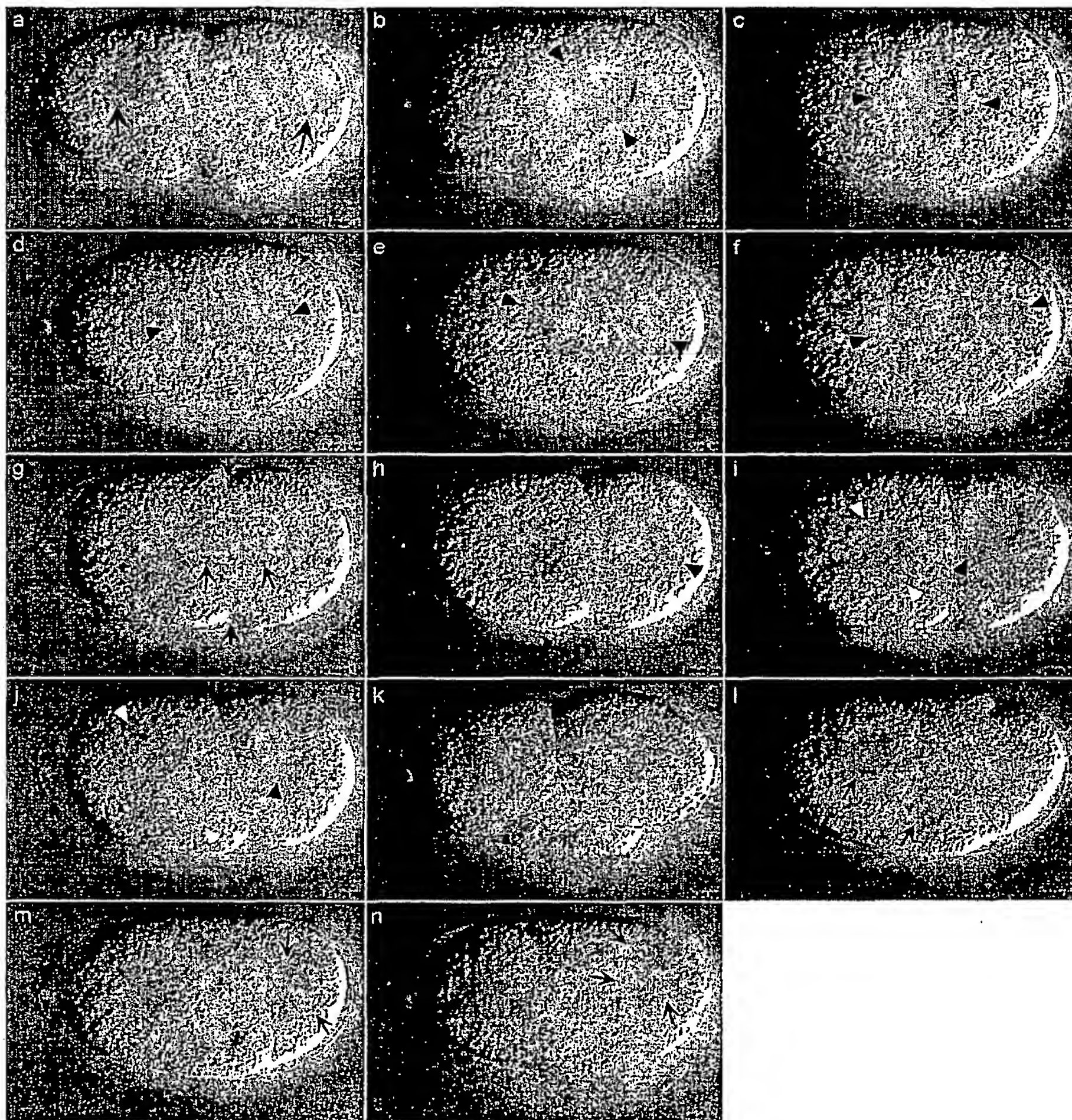


FIG. 4

Multiple Sequence Alignment of the H38K22.2a family

```

CeH38K22.2a  ---MNRLLKS--DQKTKLRQEVQWVQVTEAVSENFFAKANWNIEYAMTLYEDNENLFAGSTP
CeH38K22.2b  ---MNRLLKS--DQ-----
DmCG7427     MILONKLLKSTHROKVKKEISLHTTGEQTATFCLOONDWKFELASDNYEONBEYYRE--
MmAAF04863   ---MNRLLKS--SOKDKVROQMIFLOSSEKTAVSCHSONDWKLDVATDNFFONPELYIRESV
HsAAH09478   ---MNRLLKS--SOKDKVROQMIFLOSSEKTAVSCHSONDWKLDVATDNFFONPELYIRESV

CeH38K22.2a  QPSMDRSNLERLEFNQYVDEKDKVGEKRMGPHGINRLLTDLGYEATDRRVVVLAWKETAOQ
CeH38K22.2b  -----KTKLERLEFNQYVDEKDKVGEKRMGPHGINRLLTDLGYEATDRRVVVLAWKETAOQ
DmCG7427     ---LDRKRLEQLEFMRVDESD---PLKIGSQGVHFLLEDLDLKPD SKLVLITAWKEHAEV
MmAAF04863   KGSLEDRKKLEQHYTRYKDEQD---ENKIGIDGTOQFCDDIALDPASISVLTAWKERAAT
HsAAH09478   KGSLEDRKKLEQHYTRYKDEQD---ENKIGIDGTOQFCDDIALDPASISVLTAWKERAAT

CeH38K22.2a  QGEESLDEWVKGMTALQADTVQNRQRIDSINSGLESDEKAKTHELYLEAFNYAKSAACRN
CeH38K22.2b  QGEESLDEWVKGMTALQADTVQNRQRIDSINSGLESDEKAKTHELYLEAFNYAKSAACRN
DmCG7427     QGEESRDEFINCMCDLGIDSDKIKTKLPILEQEIIN-DAGKEKDFYHETENYAKDPGQKG
MmAAF04863   QGEESKQEFMDGNTETELGCDSTIEKLKAOIPKMEQELK-EPGREKDFYQETTFANPGQKG
HsAAH09478   QGEESKQEFMDGNTETELGCDSTIEKLKAOIPKMEQELK-EPGREKDFYQETTFANPGQKG

CeH38K22.2a  LDLETAECWDVLFGORSTIMTOWIDELWAQENAAASRLAQNVGASNAKQFKSVWISRDT
CeH38K22.2b  LDLETAECWDVLFGORSTIMTOWIDELWAQENAAASRLAQNVGASNAKQFKSVWISRDT
DmCG7427     LDEEMALAYWCLVLSGRFKFLDIICOLEEKHKRAIS-----RDT
MmAAF04863   LDEEMALAYWNLVNLGRFKFLDLNKKELLEHHKRSIP-----KDT
HsAAH09478   LDEEMALAYWNLVNLGRFKFLDLNKKELLEHHKRSIP-----KDT

CeH38K22.2a  WNEFWDEILLKSPDLSDYDDEGAWPVLLDQVVDYCRENLNYPKPGNASNDQOMETPKIAQ
CeH38K22.2b  WNEFWDEILLKSPDLSDYDDEGAWPVLLDQVVDYCRENLNYPKPGNASNDQOMETPKIAQ
DmCG7427     WNEILLDEATNIDDRMSNYDEEGAWPVLLDQVVDYCRENLNYPKPGNASNDQOMETPKIAQ
MmAAF04863   WNEILLDESSMIADDMSNYDEEGAWPVLLDQVVDYCRENLNYPKPGNASNDQOMETPKIAQ
HsAAH09478   WNEILLDESSMIADDMSNYDEEGAWPVLLDQVVDYCRENLNYPKPGNASNDQOMETPKIAQ

CeH38K22.2a  KKPGIFYFNSNLQIEFKLFQYPMKLTIFKITIHTAGTNR
CeH38K22.2b  KKPGIFYFNSNLQIEFKLFQYPMKLTIFKITIHTAGTNR
DmCG7427     SQKNISSAYQTSHTNMNYG-----
MmAAF04863   -----
HsAAH09478   -----

```

Statistics	HsAAH09478	MmAAF04863	DmCG7427
CeH38K22.2a	E-value: 1e-49 Identities: 101/275 (36%) Positives: 158/275 (56%)	E-value: 7e-49 Identities: 100/275 (36%) Positives: 157/275 (56%)	E-value: 6e-44 Identities: 104/299 (36%) Positives: 154/299 (56%)
CeH38K22.2b	E-value: 1e-35 Identities: 87/214 (36%) Positives: 118/214 (54%)	E-value: 7e-35 Identities: 77/214 (35%) Positives: 117/214 (54%)	E-value: 2e-36 Identities: 86/238 (36%) Positives: 126/238 (52%)

FIG. 5

CE61773US.ST25
SEQUENCE LISTING

<110> Cenix BioScience GmbH

<120> Eukaryotic cell division genes and their use in diagnosis and treatment of proliferative diseases

<130> CE61773US

<150> US 60/246,750

<151> 2000-11-09

<160> 26

<170> PatentIn version 3.1

<210> 1

<211> 3104

<212> DNA

<213> C. elegans

<400> 1

```
'atgaatcgac tgaagtccga tcaaaaaaca aaggtttgta aacggaaaca agacgatgaa 60
gtggagatga gtgatatgga aactgatcac aaaaagtgta gaaaacaaga aaacagtaaa 120
tttgtgcgtg tgaaaattcc attcgtcatc cattcccgtt tttctctttt tcagcattta 180
tctcgagcaa gttcgagttc tctagctcaa agcactgttc tttctgacat ttttcccaag 240
aactacgata atatcgtgag ttgtagcggg aatttcgaaa aaaaaactaa ttttgccaca 300
tcttgctgct tcgtttgtta tttcttgact agacaaattc tagctcatct agaaagctga 360
cttttctcaa aatcgttgcg agacccaaag cagaaaaatg tatctttttt aaatctacgt 420
ggaaacgcgc tccaatatta aatttcgagg ttttcccgcc aaatacctaa cgagacccaa 480
ctttggcgag cagagcgttt tgcccgcgat tttcctgcgt ctcttcaaac aatctaata 540
ctgctgctgg tttatgaaat atcaattttc ctcatTTTTT aaagctgagc aatgttttctg 600
ctcaatccta aaatttttag tagttctaata tgtgatcaac ggtttcccat ttccgatcga 660
agtcactttt taaatttctca cttttattga tttttttcgt tttgaaattc ctgatttctt 720
ccttttttagt gataagacat cagttgctga ctgtagagaa agtgtgagaa actgttagtg 780
agagagagaa aacagtttga gaaaatgaaa aatgtttttaa ataatgatata cataattatt 840
atttgatacc atttccagct ccggcagttc gtccagtgga ctcaggtcac ggaagctgtg 900
```


CE61773US.ST25

tctctcaact tcctggcaaa agctaattgg aatatacgaat acgcgatgac tctgtatttc 960
gacaatccta atctttttgc tggatcgaca ccacagccga gcgttgatag gtccaatgta 1020
cggcaattgc tgactttggc aactctacag aatgataatg ttctcacaat atttttaatt 1080
aaaatttagt tataattaga ctatagaaaa aatatttgat ttatctgaaa atacatttta 1140
tttcagttgg aataattgga aaagtgcctc caaataattg tttttgagcg cttttttaat 1200
tgttccaact gaaatcaaag ccattttcag ataaagcaaa tttttttaaa gtatatcact 1260
aagttttaat tctaaaaaag tattgggaga acatgtcaca ccgactcatt ttgttgaatt 1320
gccgacaatt gcagaattta aatttaatta tgtaaataaa agtaattttt gtagatcgag 1380
cgcctcttca atcagtatgt cgacccaaag gataaagttg gagaaaaacg aatgggaccc 1440
cacggaatca atcgtttgct cactgatctt ggctatgaag ctactgatcg ccgggttctt 1500
gtgctcgctt ggaagtttac tgcacagaca caatgtgaat tctcgttgga tgaatgggtg 1560
aaaggaatga cagctcttca agcggatact gttcaaaatt tgagacaacg aatcgattcg 1620
attaattcag gactggaatc ggataaggca aaagtacgga aaaaattaaa taactggaat 1680
tatcttccaa acttatttga aagtgggaga gcgaatttgc actttttaag aacaaattca 1740
cgcaaaacac tgtaaattga agttaattga aaaattttga tgtaaaatac agagaaaaat 1800
tacacacttt tcctcgagga gtacacgggc tgcgtaaate aacacatagc tttattgttg 1860
gttcacacca cggcagtatg ataatacaaa aaaaaattta attgaaaaat tgaaattaag 1920
atggaggaaa atgttatttc gatctggaaa taatatttat ttttgtgaaa attaataaat 1980
ataattttca gaccgaagga aaattttaat acgtttctat aataattttc gattcaaaaa 2040
tttgaattat cacaattttt aaaaacaaaa aggttctacg atcgtctcat atctaataatc 2100
ttatcagtta cagttccacg agctctacct atttgccttc aactatgcca aatccgccgc 2160
ttgccgcaat ctggatcttg aaactgccat ctgttgctgg gatgttcttt tcggacaacg 2220
atcaacaatt atgactcaat ggatcgattt tctatgggca caggagaacg cggcggcgtc 2280
tcgcctcgct cagaacgtgg gcgcttccaa tgcgaagcaa ttcaaatacg tgtggatctc 2340
tcgtgacacg tggaatctct tctgggactt tattcttctg agtaagccag atttgtcgga 2400
ttacgatgat gaaggagcat ggccagtgc tattgatcaa ttcgttgatt attgccgtga 2460

CE61773US.ST25

aaatctcaat tatccaaagc caggaaatgc gtcaaagat cagcaaattg agacaccaag 2520
 ttattattag gacaaacaat tctaaaatcc taaagggttcg tgtttcccca atttcttcct 2580
 attttcagag ttataaaata ttgcctggac gcgaaatttt gcttcaaac tacggtacca 2640
 ggtctcggca cgacaaatat tgggttaaagc cgaaaatgca cgcgccttca atgggtactg 2700
 tagtttcaca cttttcaaaa cgtaattttt tctatgacaa cagataagct ttaaaaaatc 2760
 ttgtgaaaaa cttcaaaaaa tcaaaagttt gaaggcgcac atattttaac aaaaaatggt 2820
 tcgtgccgag accggctacc gtatTTTTTTA tgcgaaattt cgcgtttgtg taatattttt 2880
 atattatacc gagaaaactc gacactttaa aggtgtggta gcgaattggg attttatttc 2940
 gaaaaatatt ctaaattatt ccaattcag aaatagcgca aaagaaacc ggaatttttt 3000
 attttaattc taatttacaa ctaatagaat tcaaattgtt tcagtatccc atgctcaaga 3060
 ctatcttcaa aataacaatt cacaccgccg gaacaaatcg ataa 3104

<210> 2
 <211> 1011
 <212> DNA
 <213> C. elegans

<400> 2
 atgaatcgac tgaagtcgga tcaaaaaaca aagctccggc agttcgtcca gtggactcag 60
 gtcacggaag ctgtgtctct caacttcctg gcaaaagcta attggaatat cgaatacgcg 120
 atgactctgt atttcgacaa tcctaattctt tttgctggat cgacaccaca gccgagcgtt 180
 gataggtcca atatcgagcg cctcttcaat cagtatgtcg acccaaagga taaagttgga 240
 gaaaaacgaa tgggacccca cggaatcaat cgtttgctca ctgatcttgg ctatgaagct 300
 actgatcgcc gggttcttgt gctcgcctgg aagtttactg cacagacaca atgtgaattc 360
 tcgttggatg aatgggtgaa aggaatgaca gctcttcaag cggatactgt tcaaaatttg 420
 agacaacgaa tcgattcgat taattcagga ctggaatcgg ataaggcaaa attccacgag 480
 ctctacctat ttgccttcaa ctatgccaaa tccgccgctt gccgcaatct ggatcttgaa 540
 actgccatct gttgctggga tgttcttttc ggacaacgat caacaattat gactcaatgg 600
 atcgattttc tatgggcaca ggagaacgcg gcggcgtctc gcctcgctca gaacgtgggc 660
 gcttccaatg cgaagcaatt caaatcgggtg tggatctctc gtgacacgtg gaatctcttc 720

CE61773US.ST25

tgggacttta ttcttctgag taagccagat ttgtcggatt acgatgatga aggagcatgg 780
 ccagtgccta ttgatcaatt cggttgattat tgccgtgaaa atctcaatta tccaaagcca 840
 ggaaatgcgt caaatgatca gcaaatggag acaccaaaaa tagcgcaaaa gaaacccgga 900
 attttttatt ttaattctaa ttacaacta atagaattca aattgtttca gtatcccatg 960
 ctcaagacta tcttcaaaat aacaattcac accgccggaa caaatcgata a 1011

<210> 3
 <211> 852
 <212> DNA
 <213> C. elegans

<400> 3
 atgaatcgac tgaagtccga tcaaaaaaca aagatcgagc gcctcttcaa tcagtatgtc 60
 gacccaaagg ataaagttgg agaaaaacga atgggacccc acggaatcaa tcgtttgctc 120
 actgatcttg gctatgaagc tactgatcgc cgggttcttg tgctcgcctg gaagtttact 180
 gcacagacac aatgtgaatt ctcggttgat gaatgggtga aaggaatgac agctcttcaa 240
 gcggatactg ttcaaaattt gagacaacga atcgattcga ttaattcagg actggaatcg 300
 gataaggcaa aattccacga gctctaccta tttgccttca actatgccaa atccgccgct 360
 tgccgcaatc tggatcttga aactgccatc tgttgctggg atgttctttt cggacaacga 420
 tcaacaatta tgactcaatg gatcgatttt ctatgggcac aggagaacgc ggcggcgtct 480
 cgcctcgctc agaacgtggg cgcttccaat gcgaagcaat tcaaatcggt gtggatctct 540
 cgtgacacgt ggaatctctt ctgggacttt attcttctga gtaagccaga tttgtcggat 600
 tacgatgatg aaggagcatg gccagtgcct attgatcaat tcgttgatta ttgccgtgaa 660
 aatctcaatt atccaaagcc aggaaatgcg tcaaatgatc agcaaatgga gacaccaaaa 720
 atagcgcaaa agaaacccgg aattttttat tttaattcta atttacaact aatagaattc 780
 aaattgtttc agtatcccat gctcaagact atcttcaaaa taacaattca caccgccgga 840
 acaaatcgat aa 852

<210> 4
 <211> 3308
 <212> DNA

CE61773US.ST25

<213> C. elegans

<400> 4

atgtcgatgg agcctcgtaa gaagcgggaac tcgattctca aggtgcggca agccgtcgaa 60
accatcgagg aaaccgtcat gaacagtggg cctagttcca caacaactaa tcgacgagtc 120
agctttcata acgtgaagca tgtcaagtca gttagagtca gtgaataatt tatcaataaa 180
ataattatth caggcagtat gacagggacc atggtaaaat tcttgacgcc acaccagtta 240
aggagaagat tactgacact attggatcag atggtattht gacgtgagtt ccatccttht 300
acgtgaaata atgaatacgt aaaaatctth ttaagaccac gtggcggaaa catggatatt 360
tccgaatctc cggcctgcac gtcctcatth caagtgttcg gcggtggtaa tctcgataaa 420
actatggata tgtctctcga aacaactatc aacgagaaca acgaaacggc gagattgtth 480
gaaaccacaa gagatccaac actattatac gaaaagatcg tcgaaaccac aacaaaagtt 540
accgagcgaa ttgttagtat gccactggat gataccttag caatgttcaa tacaacgaat 600
caagaagata aggatatgtc agttgatcgt tcagttctth tcacgattcc caaagttccg 660
aagcataacg ctacaatgaa tagaactata ccgatggacc tcgatgaatc aaaagcagcg 720
ggcggccagt gcgatgaaac ggtatgttga attaatagaa ggaaccaaat tatcttaatt 780
ttacagatga atgtgttcaa tttcacaaac ttggaagccg ctgaaatgga tacgagtaaa 840
ttagatgaaa ataataccat gaatgctatc cggattccga ttaattcaaa cgtcatgcct 900
gtagacatgg acatcactga acatcacact ttaattgaag aaaagaaaaa tgatacatth 960
gggccaagtc aactgatgga catthtcggcg ccacaagthc aagttaatga tactthtgcc 1020
atthtcaaca gtccgagaga catctgtaat aagggtthtg gtgttcctca gaatctaata 1080
aatatcgcct cgaacgtcgt acctgtggac atggacatca ctgatcaggc cgtattaaac 1140
gcggagaaga aaaatgatca attcgagaca agtcagctta tggacatthc tattccgaaa 1200
gttctagtaa atgacactat ggcgatgttc aacagcccga aacacgtcag taagagcagc 1260
atggatctcg agaaaacgat tgaagccgct gacaaatcaa cgaaataccc gagtatcgca 1320
gatgaggtgg aagatthtaga catggatatg gatatcactg aacaacaacc atgtgaggct 1380
ggtaatcagc agaacgacgg cttgcaactt caaaaggagg atttaatgga catthtcggtg 1440
attcgagatt cacctgcagt aaacgacacc atggctgtgt tccagagthc tgccagagta 1500

CE61773US.ST25

aagatcggag cggtaagttt taagcacact ttccaataaa aatgtatttc tttcagaaca 1560
actcgatcat tgattcgcag aaatctatcg tgttcgggtga cgaaatgagc attgacgaga 1620
cacaaaatga tggaaccttg acgttgccaa agtcgaatgt agaagtgact acaactaatg 1680
atgtctacac gtctctcgag cggcaagagg aaaatgcttc agaaaacgta tccatgataa 1740
acgaatcttc tgttcattcg gaaatcgaca aaaagtcggt tatgctcatc gaagaagaaa 1800
gggcttttat gcactcctcc atgattgatg tagcacaaaa gttggaagac gatggttcgt 1860
cgaagacgcc agtcatcctt gcttcacagt cagcttctct tgccactaaa gaaccatcag 1920
cccttcacaa ctcgagtgc actctcaaca attcgatgga attggacaac aatactcttc 1980
ttaaaactat gcaaattaca acgtgtgaag acattagcat ggtccatgag tctattgctg 2040
ttgaactgaa cagtaacaaa gagcaggagc aattcggaga tgagactttg cagaaaaatg 2100
gtaaatttcg tttattcaat aactctatta aaagtatggt ttagatacct cgaatactgg 2160
cgcgaatttc acattccaag gccataatga aacatcgcaa atcatgaaca atgtcgactc 2220
ggaagcagtg aacacgtcca agatttcaac atattcggct ttcaatttga gcatcaacca 2280
gtctatctct aaacgacgtc gatctcttct gaattctgct cgtgaatctc ctcgtcgtgt 2340
tgcgttggag aattctataa tgtcgatgaa tgggcaaaca atggaagctc tgacagaata 2400
tcgacagaat aaaactatgc agacgagtca agattcgatg ccgagtatga gtttgaacga 2460
ttcgggaaga gatattctcg cgatggtaag aatatctctt tgagtattga atcgaaaatg 2520
tctttcagaa tacatcagtc cgctctctc atctgaattc ttcaaaaact gctgccccag 2580
gaacaccatc attgatgtca caaatgtac aacttccacc tccatctcct caattcgaaa 2640
tgccagactt cgatccagct gtggtcaacg ttgtatatatt aacatctgaa gatccgtcca 2700
ctgaacaaca tccagaagct ctcaaatttc agcgtattgt tgaaaacgag aaaatgaaag 2760
tacaacacga gattgattct ctgaattcaa ccaatcaact ttctgctgag aaaattgata 2820
tgttgaagac taaggagctc ttgaagttta gtcatgatga gcgagaagcg attatgattg 2880
caagaaaaga cgcggaaatc aagtttttgg agcttcgtct gaaatttgca ctcgagaaaa 2940
aaattgaaag tgaccaggaa attgctgaac tagaacaagg aaattcgaaa atggctgagc 3000
agctaagagg tctcgataag atggctgtcg ttcaaaaaga actagaaaag ctgagaagtc 3060

CE61773US.ST25

ttcctccatc acgcgaagag agcgggaaaa tccgaaagga gtggatggag atgaagcaat 3120
gggaattcga ccagaâaatg aaagcactcc gaaatgtacg ctcaaacatg attgcacttc 3180
gttcagagaa aaatgctctc gaaatgaaag tcgcggaaga acacgagaag tttgcccaga 3240
ggaacgattt gaagaaaagt cgaatgctgg tgttctctaa ggctgttaag aaaattgtga 3300
acttctag 3308

<210> 5
<211> 3033
<212> DNA
<213> C. elegans

<400> 5
atgtcgatgg agcctcgtaa gaagcggaaac tcgatttctca aggtgcggca agccgtcgaa 60
accatcgagg aaaccgtcat gaacagtggg cctagttcca caacaactaa tcgacgagtc 120
agctttcata acgtgaagca tgtcaagcag tatgacaggg accatggtaa aattcttgac 180
gccacaccag ttaaggagaa gattactgac actattggat cagatgggat tttgacacca 240
cgtggcggaa acatggatat ttccgaatct ccggcctgca cgtcctcatt tcaagtgttc 300
ggcgggtggt atctcgataa aactatggat atgtctctcg aaacaactat caacgagaac 360
aacgaaacgg cgagattgtt tgaaaccaca agagatccaa cactattata cgaaaagatc 420
gtcgaaacca caacaaaagt taccgagcga attgttagta tgccactgga tgatacctta 480
gcaatgttca atacaacgaa tcaagaagat aaggatatgt cagttgatcg ttcagttctt 540
ttcacgattc ccaaagttcc gaagcataac gctacaatga atagaactat accgatggac 600
ctcgatgaat caaaagcagc gggcggccag tgcgatgaaa cgatgaatgt gttcaatttc 660
acaaacttgg aagccgctga aatggatacg agtaaattag atgaaaataa taccatgaat 720
gctatccgga ttccgattaa ttcaaacgtc atgcctgtag acatggacat cactgaacat 780
cacactttaa ttgaagaaaa gaaaaatgat acattcgggc caagtcaact gatggacatt 840
tcggcgccac aagttcaagt taatgatact ttggccattt tcaacagtcc gagagacatc 900
tgtaataagg gtttgggtgt tcctcagaat ctaataaata tcgcctcgaa cgtcgtacct 960
gtggacatgg acatcactga tcaggccgta ttaaacgcgg agaagaaaaa tgatcaattc 1020

CE61773US.ST25

gagacaagtc agcttatgga catttctatt ccgaaagttc tagtaaata cactatggcg 1080
atgttcaaca gcccgaaaca cgtcagtaag agcagcatgg atctcgagaa aacgattgaa 1140
gccgctgaca aatcaacgaa atacccgagt atcgcagatg aggtggaaga tttagacatg 1200
gatatggata tcaactgaaca acaaccatgt gaggctggta atcagcagaa cgacggcttg 1260
caacttcaaa aggaggattt aatggacatt tcggtgattc gagattcacc tgcagtaaac 1320
gacaccatgg ctgtgttcca gagtcctgcc agagtaaaga tcggagcgaa caactcgatc 1380
attgattcgc agaaatctat cgtgttcggt gacgaaatga gcattgacga gacacaaaat 1440
gatggaacct tgacgttgcc aaagtcgaat gtagaagtga ctacaactaa tgatgtctac 1500
acgtctctcg agcggcaaga ggaaaatgct tcagaaaacg tatccatgat aaacgaatct 1560
tctgttcatt cggaaatcga caaaaagtcg tttatgctca tcgaagaaga aagggtttt 1620
atgcactcct ccatgattga tgtagcacia aagttggaag acgatggttc gtcgaagacg 1680
ccagtcatcc ttgcttcaca gtcagcttct cttgccacta aagaaccatc agcccttcac 1740
aactcgagtg caactctcaa caattcgatg gaattggaca acaatactct tcttaaaact 1800
atgcaaatta caacgtgtga agacattagc atgggtccatg agtctattgc tgttgaactg 1860
aacagtaaca aagagcagga gcaattcgga gatgagactt tgcagaaaaa tgatacctcg 1920
aatactggcg cgaatttcac attccaaggc cataatgaaa catcgcaaat catgaacaat 1980
gtcgactcgg aagcagtga caggtccaag atttcaacat attcggcttt caatttgagc 2040
atcaaccagt ctatctctaa acgacgtcga tctcttctga attctgctcg tgaatctcct 2100
cgtcgtgttg cgttggagaa ttctataatg tcgatgaatg ggcaaacaat ggaagctctg 2160
acagaatate gacagaataa aactatgcag acgagtcaag attcgatgcc gagtatgagt 2220
ttgaacgatt cgggaagaga tattctcgcg atgaatacat cagtcgcgtc tcctcatctg 2280
aattcttcaa aaactgctgc cccaggaaca ccatcattga tgtcacaaaa tgtacaactt 2340
ccacctccat ctctcaatt cgaaatgcc gacttcgatc cagctgtggg caacgttgta 2400
tatttaacat ctgaagatcc gtccactgaa caacatccag aagctctcaa atttcagcgt 2460
attgttgaaa acgagaaaat gaaagtacaa cacgagattg attctctgaa ttcaaccaat 2520
caactttctg ctgagaaaat tgatatgttg aagactaagg agctcttgaa gtttagtcat 2580

CE61773US.ST25

gatgagcgag aagcgattat gattgcaaga aaagacgcgg aaatcaagtt tttggagctt 2640
 cgtctgaaat ttgcactcga gaaaaaaatt gaaagtgacc aggaaattgc tgaactagaa 2700
 caaggaaatt cgaaaatggc tgagcagcta agaggtctcg ataagatggc tgtcgttcaa 2760
 aaagaactag aaaagctgag aagtcttcct ccatcacgcg aagagagcgg gaaaatccga 2820
 aaggagtgga tggagatgaa gcaatgggaa ttcgaccaga aaatgaaagc actccgaaat 2880
 gtacgctcaa acatgattgc acttcgttca gagaaaaatg ctctcgaaat gaaagtcgcg 2940
 gaagaacacg agaagtttgc ccagaggaac gatttgaaga aaagtcgaat gctggtgttc 3000
 tctaaggctg ttaagaaaat tgtgaacttc tag 3033

<210> 6
 <211> 7097
 <212> DNA
 <213> C. elegans

<400> 6
 accgcatctc ttccaatgga tcaaccatca ttgtcatctt cgccggaaaa tcgtctaaat 60
 cccgcacctt ccgttgctga agagcatggc cacagtggac agcacgctga agaagaagaa 120
 gacaatgaca cggatgaagt atctgcaatg ccttcttttg tgcctgatga acettcgact 180
 cttgttaatt cagatcatga attgtctgat gatgctttaa agtataaaaa tgcagctgcc 240
 gaattcaaag cttttgagag aagaatggat tcggtaagaa cagccaaatc agaatgataa 300
 ttgaaatttt acatagaata gatttacgta tcaaaaatca aaacctacga atactctcta 360
 attcaaaatt taattaatta aaattaaaga tgagatcagc ttcaacaatc acaacatcac 420
 tggcaacgcc atcatcttgt gcaccatcaa actcctctga gcctcctact cggctctacac 480
 caattatgaa cgatttaggc gttggcccaa ataatcacia ttggccgtct tcaatgcaag 540
 aattatcagg aatttctctg gaaacaccac aggctcgacc gcttggcagc aatagaatta 600
 atcagcttgg taggttaata acaaaaaaaaa catgattgat tagattttta gttcgaagtg 660
 aggctcaaac ggaataagc cttttacaac accatgaaag acctactgtg accgccccat 720
 tgagacgaaa tgatatgatg aactcatcac gacagaatcc acagaatgga aatgttcaag 780
 atgaaaatcg acccgagcac gtttatgatc aaccaatata tgttcctgga tcatcactgg 840
 accgacagaa acttgaaatt gaaattcgac gtcatcgtaa cttgaacata caactgagag 900

CE61773US.ST25

aactctgccc gaaccgtgga cagtggaata tcaaatgaag acgagacccg tccaccaacc 5640
aatagctaac ggtcttcttt tcgaatatc caatggagat cttcgatggg ttaatcggca 5700
gaacgctggt aatgtaagtt ttaattggaa ttttgtcaat taaagtgacc aatttacaga 5760
tctacatata cgcagttgat aaaacagtca gaattgatct cccacatac aatatttcaa 5820
ttattcatac atttcaaagg caagttgaag tacttcgtcc tggaaataac ataacattga 5880
taagtattaa acgacgagaa gttcgaactg atttgattta tcaaaacgga atgtataaaa 5940
ctgaaatgta agattatttt cttttttaaa gttcacgga aatttcgtat ttcagcttca 6000
atagggacgg aagatatggt acgaaggatt ttagcaatca agaagtttcg agaaagtgag 6060
tctctattca tttccaatt aattattcag aaaaaccatt aaaatctcaa aactattacc 6120
cagatgttta atttaattta atttaattta ttacgataga agatatcgtt aggtagaaaa 6180
aaaaacacac acacattaat agatacaaac catcacaagt gggtacataa ataaattaca 6240
taaataaaac gaaacaaaaa taaaaaaaga gatgtgacat tttgcggcaa aaaatgtctc 6300
ggcacgataa aatttagtta aatgggaaaa ggcgtgcgcc tttaaattt actgtagttt 6360
aaaaatcgcg ttactgtcga attgttggtt gcccttttt tttttgataa aaacatgttt 6420
attagtttag aaaaaagata aataaaccaa actacaacag tctttatagg cgcacgtatt 6480
ttcacattta aaaatctgtc ctttaacgaa aaaattgtaa aatttggcgc cttcaaagag 6540
tactgtaatt tcaaactcaa tttgaaacag aattttcatc gattttcctt agttagtttt 6600
tcgatgaatt ttaatttatt cattaataaa actcaaataa gtataacgat atttttagcaa 6660
ataatatatt ttcaaacaaa acatgtttct ataatttttg tctaaccxaa aatttaggaa 6720
tatgacctca attcttcaaa aagttagtaa aacaggttta aaaccccggt ataatatttt 6780
ttgcctctga aacctatcaa attttcagat acaatcccgg tacacacaca tatcgcgaca 6840
atcaatgtcg ctacgttctc gtcactgatt acaacgattt tgagctcgtt gagccagaat 6900
tccgtcttcg ttggtatcag ggagatccga ctggtctcaa caatcagtat attctcaaga 6960
tcattggacg acctgaatgc agcgagaaaa cattgagact tgaagtgaat cttccacgt 7020
gtgaaggtag attggaaact gcagagatga taggcgataa acgtcggaaa acaactttgt 7080
tccagtggaa aaaatga 7097

CE61773US.ST25

<210> 7
<211> 3624
<212> DNA
<213> C. elegans

<400> 7
atgtcaacaa tcaccagcca aaaagggata agattattaa ctgagagacg aggggataat 60
tccctcatatc taactctcac tcttcactct ctctgctctt ctcttcattt gtcttctttt 120
tttgatattg gttgtgggtt tttgtcaccg aataataaga atgctatgaa tacatctcac 180
aattcatttt tctttttctt gcttctcttc cttttttcgt tctttttgcc gtttgccatt 240
caactttttg gtaaattgcc aaattctaag aaaatgtggg ctttcccagc aattttgagc 300
ataaatgtaa atctaatttc tagaaagttg atggtcacag tgataccaaa aataataagt 360
tctccatata ctcggacacg cctaccactg tacctctaca ctgtttccat cattatttcc 420
tgctctttat tatactggaa tcttctttac tgcaaaaatt atgactgtgt cgttgagaag 480
gaatttcgat ggggaagtac tcggcactta ctacagtact ttccggtgat agcagctccg 540
attataatgg taatatcggt ttcttggtta ataattgcaa tatattattc aagtagttca 600
tgtgttctta cattcaattt tatggaaatg ccatctgcag tactttgttc tctacttggt 660
ggatattagt ctgtaataga aattcatttt tccattgaag taaatcaagt tcaatggact 720
gatcagtggg tactgtcatc tgtgggttta ccaatcaacg attgttttaa aatcgatatt 780
ttcagggatc ttcaatactt ttatgccttt tacatgctac aattgcgttc acacttcaat 840
aatccttcca acatatttga atttccaatc ttcttcaa atcgatgaatca aaaatattat 900
gtgaactgtg atatttactc ttgctcaatt catttcatga aaaagcaaaa gaaaatgagc 960
ttttcacaag cacaagacgt atatcttcgt ctgaagcaag aaaaagaaga ggagaaacaa 1020
cgagagcgag ccgaacgaga aaagcgaaat gagacgattg cagcgacaaa taaatcaaga 1080
aagaagatga atcaggcatt ggcaaaaaga aataaaaaag gacaaccaa tctgaatgct 1140
caaatggata tggcttccga tgaaaatatc ggtgccgacg gtgaacagaa gccttctcgg 1200
ccgtttttga gaaaaggaca aggaacagca agatttagaa tggtagtttg tgcaaataca 1260
aggcttatcg aaataatata tgaagttcag cctagaaaca acaaaacatc tgctgggtgca 1320

CE61773US.ST25

cctccaacgt cggaactttc atctgcttca agtccttcta ttaatgttcc taggttttagt 1380
ctgtcgaatg ctctcccgaa ctctgcccga accgtggaca gtggaatatc aaatgaagac 1440
gagacccgtc caccaaccac cgcattctctt ccaatggatc aaccatcatt gtcattcttcg 1500
ccggaataatc gtctaaatcc cgcaccttcc gttgctgaag agcatggcca cagtggacag 1560
cacgctgaag aagaagaaga caatgacacg gatgaagtat ctgcaatgcc ttctttttgtg 1620
cctgatgaac cttcgactct tgttaattca gatcatgaat tgtctgatga tgcttttaaag 1680
tataaaaatg cagctgccga attcaaagct tttagagaga gaatggattc gatgagatca 1740
gcttcaacaa tcacaacatc actggcaacg ccatcatctt gtgcaccatc aaactcctct 1800
gagcctccta ctcggtctac accaattatg aacgatttag gcgttggccc aaataatcac 1860
aattggccgt cttcaatgca agaattatca ggaattttctc tggaaacacc acaggctcga 1920
ccgcttgga gcaatagaat taatcagctt gttcgaagtg aggctcaaac gggaataagc 1980
cttttacaac accatgaag acctactgtg accgccccat tgagacgaaa tgatatgatg 2040
aactcatcac gacagaatcc acagaatgga aatgttcaag atgaaaatcg acccgagcac 2100
gtttatgatc aaccaataca tgttcctgga tcatcactgg accgacagaa acttgaaatt 2160
gaaattcgac gtcatcgtaa cttgaacata caactgagag acactattgc tcacttgat 2220
tatgcagaag aatccgtgca caccacaaaa cgacagctcg aagaaaaaat ttccgaagtc 2280
aataatttta agaaagaact gatagaagaa ttttaagaaat gcaaaaaagg agttgaggaa 2340
gaatttgaga agaagtttga gaaaattaag gaagattatg atgaacttta cgagaaattg 2400
aagagggatc aacgagatct tgaacgagat cagaagatat tgaagaaagg aacgggagaa 2460
aggaataaag aattcacaga aacgatagcc actctccgcg acaaattaag agcatcagaa 2520
accaagaatg cacaatatcg acaggatata cgtgttcgag acgaaaagct caagaaaaaa 2580
gacgaggaaa tcgagaagct tcagaaagac ggaaaccggc taaagagcac tctacagact 2640
ttagaaaagc gcgtaaaaca attacgtact gaaaaagaac gcgacgataa agaaaaggag 2700
atgttcgcga aggttgcaat gaatcgaaaa acttcgaatc cagtgccacc agtttttgaat 2760
caaagtgttc caatttcgat aacatcaaat ggtccatcta gacatccatc atcatcttcg 2820
ttgacaacat ttagaaaacc atctacatca aatcgagaaa gaggtgttag ttgggcagat 2880

CE61773US.ST25

gaaccaaattg aacaatcatt ggaagctgta ccacaggagt ttttgatgat gccagtcaaa 294
 gaaatgccgg gaaaatttgg aaaatgcacg atctacagag attctcttgg agaaacatct 300
 aaagtgcagg atacaatagc taacgggtctt cttttcgaat attccaatgg agatcttcga 306
 tgggttaatc ggcagaacgc tgttaatatc tacatatccg cagttgataa aacagtcaga 312
 attgatctcc ccacatacaa tattttcaatt attcatacat ttcaaaggca agttgaagta 318
 ctctgtcctg gaaataacat aacattgata agtattaaac gacgagaagt tcgaactgat 324
 ttgattttatc aaaacggaat gtataaaact gaaatcttca atagggacgg aagatatgtt 330
 acgaaggatt ttagcaatca agaagtttcg agaaaataca atcccgggtac acacacatat 336
 cgcgacaatc aatgtcgcta cgttctcgtc actgattaca acgattttga gctcgttgag 342
 ccagaattcc gtcttcgttg gtatcaggga gatccgactg gtctcaacaa tcagtatatatt 348
 ctcaagatca ttggacgacc tgaatgcagc gagaaaacat tgagacttga agtgaatctt 354
 tccacgtgtg aaggtagatt ggaaactgca gagatgatag gcgataaacg tcggaaaaca 360
 actttgttcc agtggaaaaa atga 362

<210> 8
 <211> 336
 <212> PRT
 <213> C. elegans

<400> 8

Met	Asn	Arg	Leu	Lys	Ser	Asp	Gln	Lys	Thr	Lys	Leu	Arg	Gln	Phe	Val
1				5					10					15	

Gln	Trp	Thr	Gln	Val	Thr	Glu	Ala	Val	Ser	Leu	Asn	Phe	Leu	Ala	Lys
			20					25					30		

Ala	Asn	Trp	Asn	Ile	Glu	Tyr	Ala	Met	Thr	Leu	Tyr	Phe	Asp	Asn	Pro
		35					40					45			

Asn	Leu	Phe	Ala	Gly	Ser	Thr	Pro	Gln	Pro	Ser	Val	Asp	Arg	Ser	Asn
	50					55					60				

Ile	Glu	Arg	Leu	Phe	Asn	Gln	Tyr	Val	Asp	Pro	Lys	Asp	Lys	Val	Gly
65					70					75					80

CE61773US.ST25

Glu Lys Arg Met Gly Pro His Gly Ile Asn Arg Leu Leu Thr Asp Leu
85 90 95

Gly Tyr Glu Ala Thr Asp Arg Arg Val Leu Val Leu Ala Trp Lys Phe
100 105 110

Thr Ala Gln Thr Gln Cys Glu Phe Ser Leu Asp Glu Trp Val Lys Gly
115 120 125

Met Thr Ala Leu Gln Ala Asp Thr Val Gln Asn Leu Arg Gln Arg Ile
130 135 140

Asp Ser Ile Asn Ser Gly Leu Glu Ser Asp Lys Ala Lys Phe His Glu
145 150 155 160

Leu Tyr Leu Phe Ala Phe Asn Tyr Ala Lys Ser Ala Ala Cys Arg Asn
165 170 175

Leu Asp Leu Glu Thr Ala Ile Cys Cys Trp Asp Val Leu Phe Gly Gln
180 185 190

Arg Ser Thr Ile Met Thr Gln Trp Ile Asp Phe Leu Trp Ala Gln Glu
195 200 205

Asn Ala Ala Ala Ser Arg Leu Ala Gln Asn Val Gly Ala Ser Asn Ala
210 215 220

Lys Gln Phe Lys Ser Val Trp Ile Ser Arg Asp Thr Trp Asn Leu Phe
225 230 235 240

Trp Asp Phe Ile Leu Leu Ser Lys Pro Asp Leu Ser Asp Tyr Asp Asp
245 250 255

Glu Gly Ala Trp Pro Val Leu Ile Asp Gln Phe Val Asp Tyr Cys Arg
260 265 270

Glu Asn Leu Asn Tyr Pro Lys Pro Gly Asn Ala Ser Asn Asp Gln Gln
275 280 285

CE61773US.ST25

Met Glu Thr Pro Lys Ile Ala Gln Lys Lys Pro Gly Ile Phe Tyr Phe
 290 295 300

Asn Ser Asn Leu Gln Leu Ile Glu Phe Lys Leu Phe Gln Tyr Pro Met
 305 310 315 320

Leu Lys Thr Ile Phe Lys Ile Thr Ile His Thr Ala Gly Thr Asn Arg
 325 330 335

<210> 9
 <211> 283
 <212> PRT
 <213> C. elegans

<400> 9

Met Asn Arg Leu Lys Ser Asp Gln Lys Thr Lys Ile Glu Arg Leu Phe
 1 5 10 15

Asn Gln Tyr Val Asp Pro Lys Asp Lys Val Gly Glu Lys Arg Met Gly
 20 25 30

Pro His Gly Ile Asn Arg Leu Leu Thr Asp Leu Gly Tyr Glu Ala Thr
 35 40 45

Asp Arg Arg Val Leu Val Leu Ala Trp Lys Phe Thr Ala Gln Thr Gln
 50 55 60

Cys Glu Phe Ser Leu Asp Glu Trp Val Lys Gly Met Thr Ala Leu Gln
 65 70 75 80

Ala Asp Thr Val Gln Asn Leu Arg Gln Arg Ile Asp Ser Ile Asn Ser
 85 90 95

Gly Leu Glu Ser Asp Lys Ala Lys Phe His Glu Leu Tyr Leu Phe Ala
 100 105 110

Phe Asn Tyr Ala Lys Ser Ala Ala Cys Arg Asn Leu Asp Leu Glu Thr
 115 120 125

CE61773US.ST25

Ala Ile Cys Cys Trp Asp Val Leu Phe Gly Gln Arg Ser Thr Ile Met
 130 135 140

Thr Gln Trp Ile Asp Phe Leu Trp Ala Gln Glu Asn Ala Ala Ala Ser
 145 150 155 160

Arg Leu Ala Gln Asn Val Gly Ala Ser Asn Ala Lys Gln Phe Lys Ser
 165 170 175

Val Trp Ile Ser Arg Asp Thr Trp Asn Leu Phe Trp Asp Phe Ile Leu
 180 185 190

Leu Ser Lys Pro Asp Leu Ser Asp Tyr Asp Asp Glu Gly Ala Trp Pro
 195 200 205

Val Leu Ile Asp Gln Phe Val Asp Tyr Cys Arg Glu Asn Leu Asn Tyr
 210 215 220

Pro Lys Pro Gly Asn Ala Ser Asn Asp Gln Gln Met Glu Thr Pro Lys
 225 230 235 240

Ile Ala Gln Lys Lys Pro Gly Ile Phe Tyr Phe Asn Ser Asn Leu Gln
 245 250 255

Leu Ile Glu Phe Lys Leu Phe Gln Tyr Pro Met Leu Lys Thr Ile Phe
 260 265 270

Lys Ile Thr Ile His Thr Ala Gly Thr Asn Arg
 275 280

<210> 10
 <211> 1010
 <212> PRT
 <213> C. elegans

<400> 10

Met Ser Met Glu Pro Arg Lys Lys Arg Asn Ser Ile Leu Lys Val Arg
 1 5 10 15

CE61773US.ST25

Gln Ala Val Glu Thr Ile Glu Glu Thr Val Met Asn Ser Gly Pro Ser
 20 25 30

Ser Thr Thr Thr Asn Arg Arg Val Ser Phe His Asn Val Lys His Val
 35 40 45

Lys Gln Tyr Asp Arg Asp His Gly Lys Ile Leu Asp Ala Thr Pro Val
 50 55 60

Lys Glu Lys Ile Thr Asp Thr Ile Gly Ser Asp Gly Ile Leu Thr Pro
 65 70 75 80

Arg Gly Gly Asn Met Asp Ile Ser Glu Ser Pro Ala Cys Thr Ser Ser
 85 90 95

Phe Gln Val Phe Gly Gly Gly Asn Leu Asp Lys Thr Met Asp Met Ser
 100 105 110

Leu Glu Thr Thr Ile Asn Glu Asn Asn Glu Thr Ala Arg Leu Phe Glu
 115 120 125

Thr Thr Arg Asp Pro Thr Leu Leu Tyr Glu Lys Ile Val Glu Thr Thr
 130 135 140

Thr Lys Val Thr Glu Arg Ile Val Ser Met Pro Leu Asp Asp Thr Leu
 145 150 155 160

Ala Met Phe Asn Thr Thr Asn Gln Glu Asp Lys Asp Met Ser Val Asp
 165 170 175

Arg Ser Val Leu Phe Thr Ile Pro Lys Val Pro Lys His Asn Ala Thr
 180 185 190

Met Asn Arg Thr Ile Pro Met Asp Leu Asp Glu Ser Lys Ala Ala Gly
 195 200 205

Gly Gln Cys Asp Glu Thr Met Asn Val Phe Asn Phe Thr Asn Leu Glu
 210 215 220

CE61773US.ST25

Ala Ala Glu Met Asp Thr Ser Lys Leu Asp Glu Asn Asn Thr Met Asn
 225 230 235 240

Ala Ile Arg Ile Pro Ile Asn Ser Asn Val Met Pro Val Asp Met Asp
 245 250 255

Ile Thr Glu His His Thr Leu Ile Glu Glu Lys Lys Asn Asp Thr Phe
 260 265 270

Gly Pro Ser Gln Leu Met Asp Ile Ser Ala Pro Gln Val Gln Val Asn
 275 280 285

Asp Thr Leu Ala Ile Phe Asn Ser Pro Arg Asp Ile Cys Asn Lys Gly
 290 295 300

Leu Gly Val Pro Gln Asn Leu Ile Asn Ile Ala Ser Asn Val Val Pro
 305 310 315 320

Val Asp Met Asp Ile Thr Asp Gln Ala Val Leu Asn Ala Glu Lys Lys
 325 330 335

Asn Asp Gln Phe Glu Thr Ser Gln Leu Met Asp Ile Ser Ile Pro Lys
 340 345 350

Val Leu Val Asn Asp Thr Met Ala Met Phe Asn Ser Pro Lys His Val
 355 360 365

Ser Lys Ser Ser Met Asp Leu Glu Lys Thr Ile Glu Ala Ala Asp Lys
 370 375 380

Ser Thr Lys Tyr Pro Ser Ile Ala Asp Glu Val Glu Asp Leu Asp Met
 385 390 395 400

Asp Met Asp Ile Thr Glu Gln Gln Pro Cys Glu Ala Gly Asn Gln Gln
 405 410 415

Asn Asp Gly Leu Gln Leu Gln Lys Glu Asp Leu Met Asp Ile Ser Val
 420 425 430

CE61773US.ST25

Ile Arg Asp Ser Pro Ala Val Asn Asp Thr Met Ala Val Phe Gln Ser
435 440 445

Pro Ala Arg Val Lys Ile Gly Ala Asn Asn Ser Ile Ile Asp Ser Gln
450 455 460

Lys Ser Ile Val Phe Gly Asp Glu Met Ser Ile Asp Glu Thr Gln Asn
465 470 475 480

Asp Gly Thr Leu Thr Leu Pro Lys Ser Asn Val Glu Val Thr Thr Thr
485 490 495

Asn Asp Val Tyr Thr Ser Leu Glu Arg Gln Glu Glu Asn Ala Ser Glu
500 505 510

Asn Val Ser Met Ile Asn Glu Ser Ser Val His Ser Glu Ile Asp Lys
515 520 525

Lys Ser Phe Met Leu Ile Glu Glu Glu Arg Ala Phe Met His Ser Ser
530 535 540

Met Ile Asp Val Ala Gln Lys Leu Glu Asp Asp Gly Ser Ser Lys Thr
545 550 555 560

Pro Val Ile Leu Ala Ser Gln Ser Ala Ser Leu Ala Thr Lys Glu Pro
565 570 575

Ser Ala Leu His Asn Ser Ser Ala Thr Leu Asn Asn Ser Met Glu Leu
580 585 590

Asp Asn Asn Thr Leu Leu Lys Thr Met Gln Ile Thr Thr Cys Glu Asp
595 600 605

Ile Ser Met Val His Glu Ser Ile Ala Val Glu Leu Asn Ser Asn Lys
610 615 620

Glu Gln Glu Gln Phe Gly Asp Glu Thr Leu Gln Lys Asn Asp Thr Ser
625 630 635 640

CE61773US.ST25

Asn Thr Gly Ala Asn Phe Thr Phe Gln Gly His Asn Glu Thr Ser Gln
 645 650 655

Ile Met Asn Asn Val Asp Ser Glu Ala Val Asn Thr Ser Lys Ile Ser
 660 665 670

Thr Tyr Ser Ala Phe Asn Leu Ser Ile Asn Gln Ser Ile Ser Lys Arg
 675 680 685

Arg Arg Ser Leu Leu Asn Ser Ala Arg Glu Ser Pro Arg Arg Val Ala
 690 695 700

Leu Glu Asn Ser Ile Met Ser Met Asn Gly Gln Thr Met Glu Ala Leu
 705 710 715 720

Thr Glu Tyr Arg Gln Asn Lys Thr Met Gln Thr Ser Gln Asp Ser Met
 725 730 735

Pro Ser Met Ser Leu Asn Asp Ser Gly Arg Asp Ile Leu Ala Met Asn
 740 745 750

Thr Ser Val Arg Ser Pro His Leu Asn Ser Ser Lys Thr Ala Ala Pro
 755 760 765

Gly Thr Pro Ser Leu Met Ser Gln Asn Val Gln Leu Pro Pro Pro Ser
 770 775 780

Pro Gln Phe Glu Met Pro Asp Phe Asp Pro Ala Val Val Asn Val Val
 785 790 795 800

Tyr Leu Thr Ser Glu Asp Pro Ser Thr Glu Gln His Pro Glu Ala Leu
 805 810 815

Lys Phe Gln Arg Ile Val Glu Asn Glu Lys Met Lys Val Gln His Glu
 820 825 830

Ile Asp Ser Leu Asn Ser Thr Asn Gln Leu Ser Ala Glu Lys Ile Asp
 835 840 845

CE61773US.ST25

Met Leu Lys Thr Lys Glu Leu Leu Lys Phe Ser His Asp Glu Arg Glu
 850 855 860

Ala Ile Met Ile Ala Arg Lys Asp Ala Glu Ile Lys Phe Leu Glu Leu
 865 870 875 880

Arg Leu Lys Phe Ala Leu Glu Lys Lys Ile Glu Ser Asp Gln Glu Ile
 885 890 895

Ala Glu Leu Glu Gln Gly Asn Ser Lys Met Ala Glu Gln Leu Arg Gly
 900 905 910

Leu Asp Lys Met Ala Val Val Gln Lys Glu Leu Glu Lys Leu Arg Ser
 915 920 925

Leu Pro Pro Ser Arg Glu Glu Ser Gly Lys Ile Arg Lys Glu Trp Met
 930 935 940

Glu Met Lys Gln Trp Glu Phe Asp Gln Lys Met Lys Ala Leu Arg Asn
 945 950 955 960

Val Arg Ser Asn Met Ile Ala Leu Arg Ser Glu Lys Asn Ala Leu Glu
 965 970 975

Met Lys Val Ala Glu Glu His Glu Lys Phe Ala Gln Arg Asn Asp Leu
 980 985 990

Lys Lys Ser Arg Met Leu Val Phe Ser Lys Ala Val Lys Lys Ile Val
 995 1000 1005

Asn Phe
 1010

<210> 11
 <211> 1207
 <212> PRT
 <213> C. elegans

<400> 11

Met Ser Thr Ile Thr Ser Gln Lys Gly Ile Arg Leu Leu Thr Glu Arg

CE61773US.ST25

1		5		10		15
Arg	Gly	Asp	Asn	Ser	Leu	Cys
		20			25	30
Ser	Ser	Pro	His	Leu	Ser	Leu
		35			40	45
Ser	Pro	Asn	Asn	Lys	Asn	Phe
	50				55	60
Phe	Phe	Leu	Leu	Leu	Phe	Leu
65				70		75
Gln	Leu	Phe	Gly	Lys	Leu	Pro
				85		90
Ala	Ile	Leu	Ser	Ile	Asn	Val
		100			105	110
Thr	Val	Ile	Pro	Lys	Ile	Ile
		115			120	125
Pro	Leu	Tyr	Leu	Tyr	Thr	Val
	130				135	140
Tyr	Trp	Asn	Leu	Leu	Tyr	Cys
145				150		155
Glu	Phe	Arg	Trp	Gly	Ser	Thr
				165		170
Ile	Ala	Ala	Pro	Ile	Ile	Met
			180			185
Ala	Ile	Tyr	Tyr	Ser	Ser	Ser
		195			200	205
Glu	Met	Pro	Ser	Ala	Val	Leu

210 215 220

Cys Ala Asn Thr Arg Leu Ile Glu Ile Ile Tyr Glu Val Gln Pro Arg

CE61773US.ST25

420

425

430

Asn Asn Lys Thr Ser Ala Gly Ala Pro Pro Thr Ser Glu Leu Ser Ser
 435 440 445

Ala Ser Ser Pro Ser Ile Asn Val Pro Arg Phe Ser Leu Ser Asn Ala
 450 455 460

Leu Pro Asn Ser Ala Arg Thr Val Asp Ser Gly Ile Ser Asn Glu Asp
 465 470 475 480

Glu Thr Arg Pro Pro Thr Thr Ala Ser Leu Pro Met Asp Gln Pro Ser
 485 490 495

Leu Ser Ser Ser Pro Glu Asn Arg Leu Asn Pro Ala Pro Ser Val Ala
 500 505 510

Glu Glu His Gly His Ser Gly Gln His Ala Glu Glu Glu Glu Asp Asn
 515 520 525

Asp Thr Asp Glu Val Ser Ala Met Pro Ser Phe Val Pro Asp Glu Pro
 530 535 540

Ser Thr Leu Val Asn Ser Asp His Glu Leu Ser Asp Asp Ala Leu Lys
 545 550 555 560

Tyr Lys Asn Ala Ala Ala Glu Phe Lys Ala Phe Glu Arg Arg Met Asp
 565 570 575

Ser Met Arg Ser Ala Ser Thr Ile Thr Thr Ser Leu Ala Thr Pro Ser
 580 585 590

Ser Cys Ala Pro Ser Asn Ser Ser Glu Pro Pro Thr Arg Ser Thr Pro
 595 600 605

Ile Met Asn Asp Leu Gly Val Gly Pro Asn Asn His Asn Trp Pro Ser
 610 615 620

Ser Met Gln Glu Leu Ser Gly Ile Ser Leu Glu Thr Pro Gln Ala Arg

CE61773US.ST25

625					630					635					640
Pro	Leu	Gly	Ser	Asn	Arg	Ile	Asn	Gln	Leu	Val	Arg	Ser	Glu	Ala	Gln
				645					650					655	
Thr	Gly	Ile	Ser	Leu	Leu	Gln	His	His	Glu	Arg	Pro	Thr	Val	Thr	Ala
			660					665					670		
Pro	Leu	Arg	Arg	Asn	Asp	Met	Met	Asn	Ser	Ser	Arg	Gln	Asn	Pro	Gln
		675					680					685			
Asn	Gly	Asn	Val	Gln	Asp	Glu	Asn	Arg	Pro	Glu	His	Val	Tyr	Asp	Gln
	690					695					700				
Pro	Ile	His	Val	Pro	Gly	Ser	Ser	Leu	Asp	Arg	Gln	Lys	Leu	Glu	Ile
705					710					715					720
Glu	Ile	Arg	Arg	His	Arg	Asn	Leu	Asn	Ile	Gln	Leu	Arg	Asp	Thr	Ile
				725					730					735	
Ala	His	Leu	Asp	Tyr	Ala	Glu	Glu	Ser	Val	His	Thr	Thr	Lys	Arg	Gln
			740					745					750		
Leu	Glu	Glu	Lys	Ile	Ser	Glu	Val	Asn	Asn	Phe	Lys	Lys	Glu	Leu	Ile
		755					760					765			
Glu	Glu	Phe	Lys	Lys	Cys	Lys	Lys	Gly	Val	Glu	Glu	Glu	Phe	Glu	Lys
	770					775					780				
Lys	Phe	Glu	Lys	Ile	Lys	Glu	Asp	Tyr	Asp	Glu	Leu	Tyr	Glu	Lys	Leu
785					790					795					800
Lys	Arg	Asp	Gln	Arg	Asp	Leu	Glu	Arg	Asp	Gln	Lys	Ile	Leu	Lys	Lys
				805					810					815	
Gly	Thr	Gly	Glu	Arg	Asn	Lys	Glu	Phe	Thr	Glu	Thr	Ile	Ala	Thr	Leu
			820					825					830		
Arg	Asp	Lys	Leu	Arg	Ala	Ser	Glu	Thr	Lys	Asn	Ala	Gln	Tyr	Arg	Gln

CE61773US.ST25

835

840

845

Asp Ile Arg Val Arg Asp Glu Lys Leu Lys Lys Lys Asp Glu Glu Ile
 850 855 860

Glu Lys Leu Gln Lys Asp Gly Asn Arg Leu Lys Ser Thr Leu Gln Thr
 865 870 875 880

Leu Glu Lys Arg Val Lys Gln Leu Arg Thr Glu Lys Glu Arg Asp Asp
 885 890 895

Lys Glu Lys Glu Met Phe Ala Lys Val Ala Met Asn Arg Lys Thr Ser
 900 905 910

Asn Pro Val Pro Pro Val Leu Asn Gln Ser Val Pro Ile Ser Ile Thr
 915 920 925

Ser Asn Gly Pro Ser Arg His Pro Ser Ser Ser Ser Leu Thr Thr Phe
 930 935 940

Arg Lys Pro Ser Thr Ser Asn Arg Glu Arg Gly Val Ser Trp Ala Asp
 945 950 955 960

Glu Pro Asn Glu Gln Ser Leu Glu Ala Val Pro Gln Glu Phe Leu Met
 965 970 975

Met Pro Val Lys Glu Met Pro Gly Lys Phe Gly Lys Cys Thr Ile Tyr
 980 985 990

Arg Asp Ser Leu Gly Glu Thr Ser Lys Val Thr Asp Thr Ile Ala Asn
 995 1000 1005

Gly Leu Leu Phe Glu Tyr Ser Asn Gly Asp Leu Arg Trp Val Asn
 1010 1015 1020

Arg Gln Asn Ala Val Asn Ile Tyr Ile Ser Ala Val Asp Lys Thr
 1025 1030 1035

Val Arg Ile Asp Leu Pro Thr Tyr Asn Ile Ser Ile Ile His Thr

CE61773US.ST25

1040 1045 1050
 Phe Gln Arg Gln Val Glu Val Leu Arg Pro Gly Asn Asn Ile Thr
 1055 1060 1065
 Leu Ile Ser Ile Lys Arg Arg Glu Val Arg Thr Asp Leu Ile Tyr
 1070 1075 1080
 Gln Asn Gly Met Tyr Lys Thr Glu Ile Phe Asn Arg Asp Gly Arg
 1085 1090 1095
 Tyr Val Thr Lys Asp Phe Ser Asn Gln Glu Val Ser Arg Lys Tyr
 1100 1105 1110
 Asn Pro Gly Thr His Thr Tyr Arg Asp Asn Gln Cys Arg Tyr Val
 1115 1120 1125
 Leu Val Thr Asp Tyr Asn Asp Phe Glu Leu Val Glu Pro Glu Phe
 1130 1135 1140
 Arg Leu Arg Trp Tyr Gln Gly Asp Pro Thr Gly Leu Asn Asn Gln
 1145 1150 1155
 Tyr Ile Leu Lys Ile Ile Gly Arg Pro Glu Cys Ser Glu Lys Thr
 1160 1165 1170
 Leu Arg Leu Glu Val Asn Leu Ser Thr Cys Glu Gly Thr Leu Glu
 1175 1180 1185
 Thr Ala Glu Met Ile Gly Asp Lys Arg Arg Lys Thr Thr Leu Phe
 1190 1195 1200
 Gln Trp Lys Lys
 1205

<210> 12
 <211> 780
 <212> DNA
 <213> homo sapiens

CE61773US.ST25

<400> 12
 atgaacaagt tgaaatcatc gcagaaggat aaagttcgtc agtttatgat cttcacacaa 60
 tctagtga aaacagcagt aagttgtctt tctcaaatg actggaagtt agatgttgca 120
 acagataatt ttttccaaaa tcttgaactt tatatacgag agagtgtaaa aggatcattg 180
 gacaggaaga agttagaaca gctgtacaat agatacaaag accctcaaga tgagaataaa 240
 attggaatag atggcataca gcagttctgt gatgacctgg cactcgatcc agccagcatt 300
 agtgtgttga ttattgcgtg gaagttcaga gcagcaacac agtgcgagtt ctccaaacag 360
 gagttcatgg atggcatgac agaattagga tgtgacagca tagaacaact aaaggcccag 420
 ataccaaga tggaacaaga attgaaagaa ccaggacgat ttaaggattt ttaccagttt 480
 acttttaatt ttgcaaagaa tccaggacaa aaaggattag atctagaaat ggccattgcc 540
 tactggaact tagtgcttaa tggaagattt aaattcttag acttatggaa taaatttttg 600
 ttggaacatc ataaacgatc aataccaaaa gacacttgga atcttctttt agacttcagt 660
 acgatgattg cagatgacat gtctaattat gatgaagaag gagcatggcc tgttcttatt 720
 gatgactttg tggaatttgc acgccctcaa attgctggga caaaaagtac aacagtgtag 780

<210> 13
 <211> 259
 <212> PRT
 <213> homo sapiens

<400> 13

Met Asn Lys Leu Lys Ser Ser Gln Lys Asp Lys Val Arg Gln Phe Met
 1 5 10 15

Ile Phe Thr Gln Ser Ser Glu Lys Thr Ala Val Ser Cys Leu Ser Gln
 20 25 30

Asn Asp Trp Lys Leu Asp Val Ala Thr Asp Asn Phe Phe Gln Asn Pro
 35 40 45

Glu Leu Tyr Ile Arg Glu Ser Val Lys Gly Ser Leu Asp Arg Lys Lys
 50 55 60

Leu Glu Gln Leu Tyr Asn Arg Tyr Lys Asp Pro Gln Asp Glu Asn Lys

[illegible]

CE61773US.ST25

<211> 258

<212> PRT

<213> Homo sapiens

<400> 14

Ser Lys Gln Glu Phe Met Asp Gly Met Thr Glu Leu Gly Cys Asp Ser
 1 5 10 15

Ile Glu Gln Leu Lys Ala Gln Ile Pro Lys Met Glu Gln Glu Leu Lys
 20 25 30

Glu Pro Gly Arg Phe Lys Asp Phe Tyr Gln Phe Thr Phe Asn Phe Ala
 35 40 45

Lys Asn Pro Gly Gln Lys Gly Leu Asp Leu Glu Asp Arg Lys Lys Leu
 50 55 60

Glu Gln Leu Tyr Asn Arg Tyr Lys Asp Pro Gln Asp Glu Asn Lys Ile
 65 70 75 80

Gly Ile Asp Gly Ile Gln Gln Phe Cys Asp Asp Leu Ala Leu Asp Pro
 85 90 95

Ala Ser Ile Ser Val Leu Ile Ile Ala Trp Lys Phe Arg Ala Ala Thr
 100 105 110

Gln Cys Glu Phe Ser Lys Gln Glu Phe Met Asp Gly Met Thr Glu Leu
 115 120 125

Gly Cys Asp Ser Ile Glu Gln Leu Lys Ala Gln Ile Pro Lys Met Glu
 130 135 140

Gln Glu Leu Lys Glu Pro Gly Arg Phe Lys Asp Phe Tyr Gln Phe Thr
 145 150 155 160

Phe Asn Phe Ala Lys Asn Pro Gly Gln Lys Gly Leu Asp Leu Glu Met
 165 170 175

Ala Ile Ala Tyr Trp Asn Leu Val Leu Asn Gly Arg Phe Lys Phe Leu
 180 185 190

CE61773US.ST25

Asp Leu Trp Asn Lys Phe Leu Leu Glu His His Lys Arg Ser Ile Pro
 195 200 205

Lys Asp Thr Trp Asn Leu Leu Leu Asp Phe Ser Thr Met Ile Ala Asp
 210 215 220

Asp Met Ser Asn Tyr Asp Glu Glu Gly Ala Trp Pro Val Leu Ile Asp
 225 230 235 240

Asp Phe Val Glu Phe Ala Arg Pro Gln Ile Ala Gly Thr Lys Ser Thr
 245 250 255

Thr Val

<210> 15
 <211> 19
 <212> DNA
 <213> artificial sequence

<220>
 <223> T7 polymerase promoter sequence (example 1)

<400> 15
 taatacgact cactatagg 19

<210> 16
 <211> 19
 <212> DNA
 <213> artificial sequence

<220>
 <223> T3 polymerase promoter sequence

<400> 16
 aattaaccct cactaaagg 19

<210> 17
 <211> 19
 <212> DNA
 <213> artificial sequence

<220>

CE61773US.ST25

<223> oligonucleotide for PCR amplification (example 3)

<400> 17

tcaatcagta tgtcgaccc

19

<210> 18

<211> 19

<212> DNA

<213> artificial sequence

<220>

<223> oligonucleotide for PCR amplification (example 3)

<400> 18

ggaagaaatt ggggaaaca

19

<210> 19

<211> 19

<212> DNA

<213> artificial sequence

<220>

<223> oligonucleotide for PCR amplification (example 3)

<400> 19

atcgagcgcc tcttcaatc

19

<210> 20

<211> 19

<212> DNA

<213> artificial sequence

<220>

<223> oligonucleotide for PCR amplification (example 3)

<400> 20

tggtgtctcc atttgctga

19

<210> 21

<211> 19

<212> DNA

<213> artificial sequence

<220>

<223> oligonucleotide for PCR amplification (example 4)

<400> 21

atctgaagat ccgtccact

19

CE61773US.ST25

<210> 22
<211> 19
<212> DNA
<213> artificial sequence

<220>
<223> oligonucleotide for PCR amplification (example 4)

<400> 22
atgcacaatg ggtatTTTT 19

<210> 23
<211> 23
<212> DNA
<213> artificial sequence

<220>
<223> oligonucleotide for PCR amplification (example 5; forward
primer to generate dsRNA 305A12)

<400> 23
ttcgtctcga acacgtatat cct 23

<210> 24
<211> 23
<212> DNA
<213> artificial sequence

<220>
<223> oligonucleotide for PCR amplification (example 5; reverse
primer to generate dsRNA 305A12)

<400> 24
gaaagaagat gaatcaggca ttg 23

<210> 25
<211> 23
<212> DNA
<213> artificial sequence

<220>
<223> oligonucleotide for PCR amplification (example 5; forward
primer to generate dsRNA 341G5)

<400> 25
ctgcaaaaat tatgactgtg tcg 23

CE61773US.ST25

<210> 26

<211> 21

<212> DNA

<213> artificial sequence

<220>

<223> oligonucleotide for PCR amplification (example 5; reverse primer to generate dsRNA 341G5)

<400> 26

agcattcaga tttggttgtc c

21